GLORI 2020

Wednesday 19th February

Glasgow University Student Union
32 University Avenue, G12 8LX

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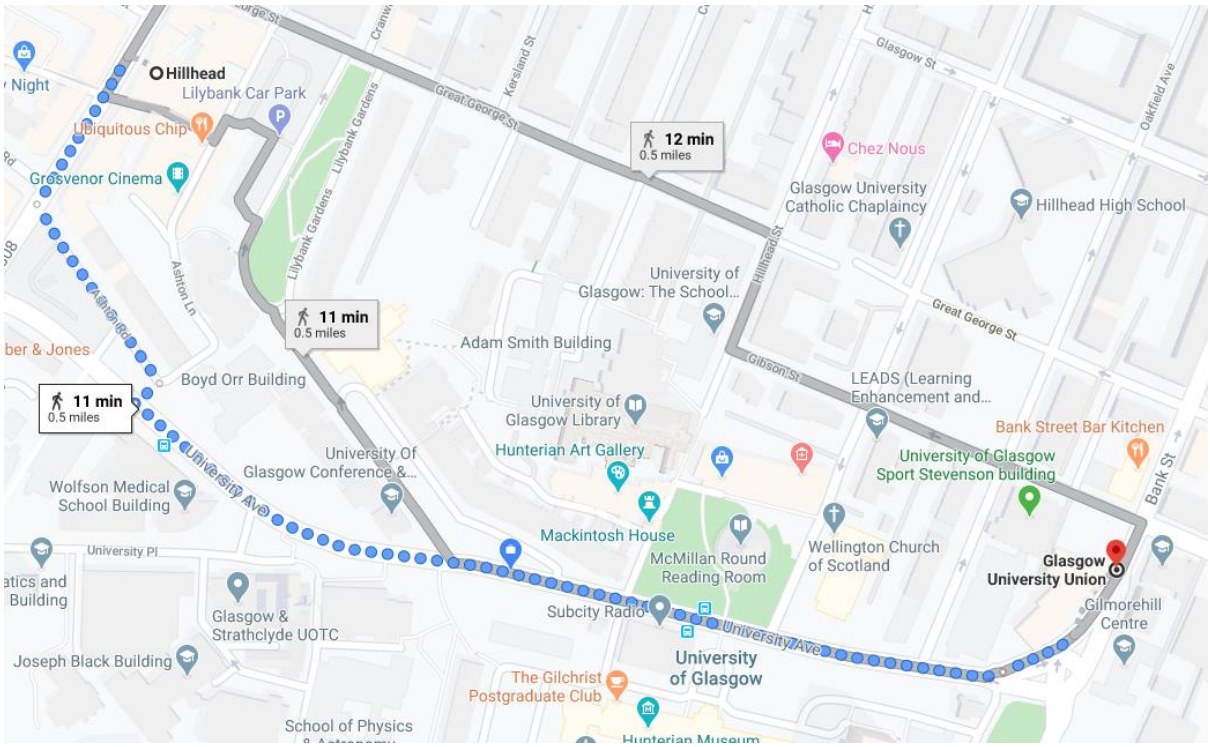
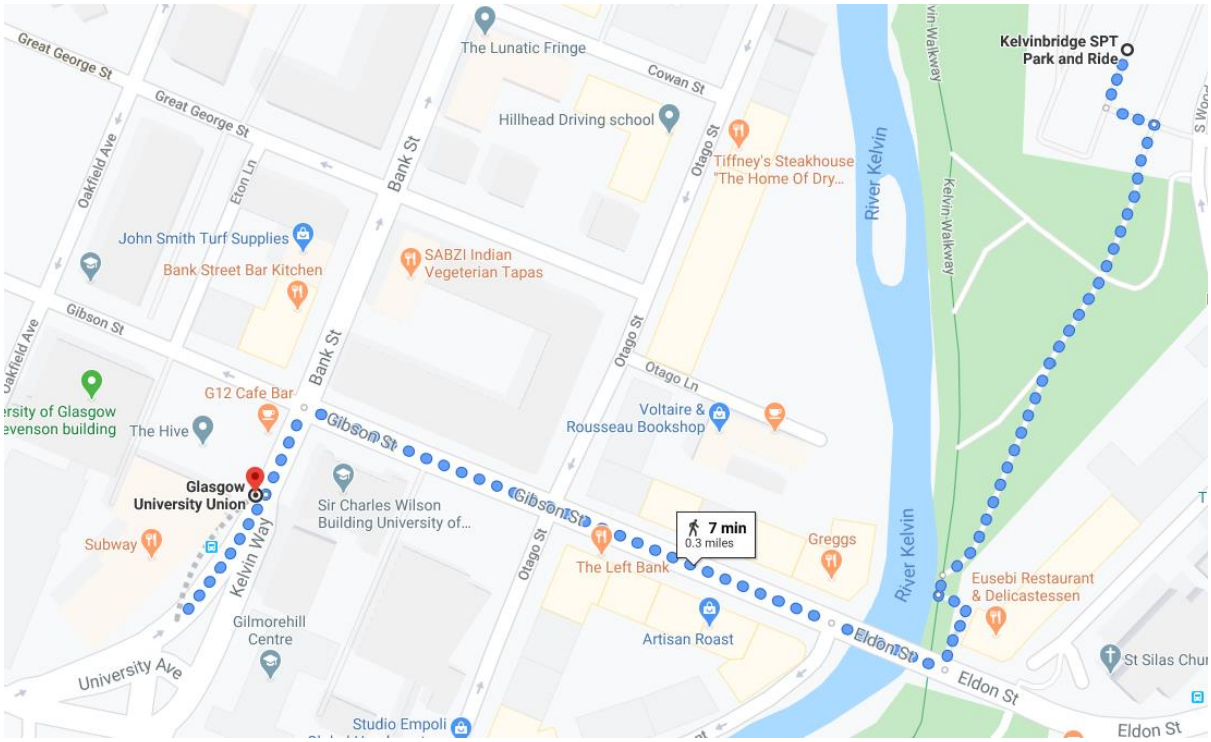
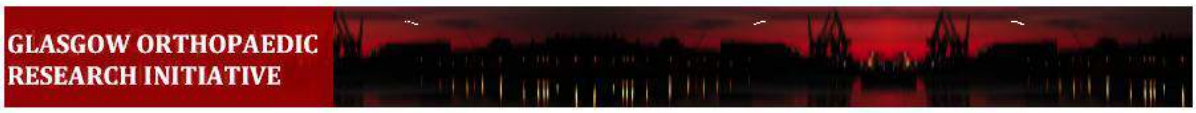
The Glasgow Orthopaedic Research Initiative (GLORI) has been established to encourage collaboration between the basic sciences, applied sciences, engineering and clinic. This has the aim of bringing the latest ideas in basic materials research into use to deliver the next-generation of orthopaedic care. It combines expertise from orthopaedic surgeons, plastic surgeons, biologists, engineers and chemists.

Directions from Hillhead Subway:



Directions from Kelvinbridge Subway:





GLORI 2020

Meeting of the Glasgow Orthopaedic Research Initiative on Wednesday 19th February

Glasgow University Union, 32 University Avenue, G12 8QQ

PROGRAMME

09:00	**Registration/coffee**
Session 1 – Chairs: Mark Sprott & Simon Clark	
09:30	Welcome from (Dominic/Matt)
09:35	**Speaker**: Dr Sylvie Coupaud *(35+5)* **Talk title**: What do astronauts and paraplegics have in common? **Affiliation**: Biomedical Engineering, University of Strathclyde
10:15	**Speaker:** Mr William Marshall *(15+5)* **Talk title:** Clinical application of healiOst in dogs and cats (2018-2020): selected short stories **Affiliation:** Small Animal Clinical Sciences, University of Glasgow
10:35	**Speaker**: Ms Laila Damiati *(7+3)* **Talk title**: Biofunctional and antibacterial titanium nanotopography for orthopaedic implants **Affiliation**: Centre for the Cellular Microenvironment, University of Glasgow
10:45	**Tea/coffee**
Session 2 – Chairs: Virginia Llopis-Hernandez & Matt Walker	
11:00	**Speaker**: Professor Richie Gill *(35+5)* **Talk title**: In Silico Trials and Machine Learning for Orthopaedics and Trauma **Affiliation**: Department of Mechanical Engineering, University of Bath
11:40	**Speaker**: Mr Robert Silverwood *(15+5)* **Talk title**: MicroRNA expression and function in fragility fractures **Affiliation**: NHS, Glasgow
12:00	**Speaker:** Mr Ian Kennedy *(15+5)* **Title:** Nanoscale vibration to modulate osteoclastogenesis **Affiliation:** Department of Trauma and Orthopaedics, Queen Elizabeth University Hospital
12:20	**Speaker**: Ms Caroline Busch *(7+3)* **Talk title**: 3D modelling of the bone marrow niche as a potential platform for testing new therapeutic approaches in Chronic Myeloid Leukaemia **Affiliation**: Centre for the Cellular Microenvironment, University of Glasgow
12:30	LI-COR presentation, Shula Dawson
12:35	Biocomposites presentation, Bethany Loughlin

12:40	PromoCell presentation, Andrew Woods
12:45	**Posters and lunch**
Session 3 – Robert Silverwood & Monica Tsimbouri	
13:45	**Speaker**: Dr Donal Wall *(35+5)* **Talk title**: Microbiome-derived carnitine mimics as novel mediators of gut-brain axis communication **Affiliation**: Institute of Infection, Immunity and Inflammation, University of Glasgow
14:25	**Speaker:** Mr David Shields *(15+5)* **Title:** Bone Defects: Opportunities and Obstacles **Affiliation:** Orthopaedic Registrar, NHS Greater Glasgow and Clyde
14:45	**Speaker:** Dr Mark Williams *(15+5)* **Talk title**: Establishing a unique 3D co-culture system to model and therapeutically target leukaemia-bone marrow microenvironment interactions in Acute Myeloid Leukaemia **Affiliation**: Glasgow Caledonian University
15:05	**Speaker:** Dr Jennifer Z Paxton *(15+5)* **Talk title:** Making Connections: Using anatomy to guide tissue-engineered design **Affiliation:** The University of Edinburgh
15:25	**Speaker**: Ms Gillian Higgins *(15+5)* **Talk title**: Superparamagnetic Iron oxide Nanoparticle labelling of therapeutic cells **Affiliation**: Canniesburn Plastic and Reconstructive Surgery Department, Glasgow Royal Infirmary
15:45	**Speaker:** Dr Hannah Donnelly *(15+5)* **Talk title:** Mechanistic and metabolic insights into bioengineering the bone marrow niche *in vitro* **Affiliation:** Centre for the Cellular Microenvironment, University of Glasgow
16:05	**Tea/coffee**
	Posters and prizes
16:30	**Meeting ends**

Oral presentations

WHAT DO ASTRONAUTS & PARAPLEGICS HAVE IN COMMON?

Sylvie Coupaud [1,2] & Mariel Purcell [2]

1 Department of Biomedical Engineering, Wolfson Centre, Strathclyde University, UK
2 Scottish Centre for Innovation in Spinal Cord Injury, Queen Elizabeth National Spinal Injuries Unit, Glasgow

Why should anyone worry about their bone health if they have been living in space for less than a year, especially if they are young and fit? The same question can be directed at paraplegics who have suffered a complete spinal cord injury for this relatively short period in the skeleton's lifetime. And yet, there are astronauts and paraplegics for whom 11 months of disuse from microgravity exposure or muscle paralysis, respectively, are sufficient to lead to significant decreases in bone mineral density (BMD) in the lower limb long bones. Although there is considerable inter-subject variability in patterns of bone loss, those individuals with the most rapid and extensive decreases in BMD are of particular concern, as low BMD is associated with an increased risk of fragility fractures in the lower-limb long bones. We present detailed longitudinal imaging data quantifying the extent of that bone loss in the femur and tibia in patients with motor-complete spinal cord injury, and compare these with published data from astronauts who have returned from long-term space missions. To complement imaging outcome measures, data from biomarkers of bone formation and resorption in serum/plasma are available from studies in: i) patients with spinal cord injuries, ii) subjects on bedrest studies, and iii) astronauts returning from long-term space missions. The rapid decline in BMD in astronauts and paraplegics – compared to the slower time-course seen in post-menopausal osteoporosis – is not easily mitigated. Those at risk of rapid and significant bone loss may be offered early treatment interventions (physical or pharmacological). However, exercise/rehabilitation interventions have shown limited efficacy in attenuating bone loss in the early stages of disuse osteoporosis, let alone reversing it once it has already occurred. The effects of available interventions and their limitations will be discussed, as well as avenues for current and further research.

CLINICAL APPLICATION OF HEALIOST IN DOGS AND CATS (2018-2020): SELECTED SHORT STORIES

William Marshall [1], Cristina Gonzalez-Garcia[2], Sara Trujillo Munoz[2], Peter Childs[2], David Shields[2,3], Elena Addison[1], Sandra Corr[1], Matt Dalby [2], Manuel Salmeron-Sanchez [2]

1 Small Animal Hospital, School of Veterinary Medicine, University of Glasgow, UK
2 Centre for the Cellular Microenvironment, University of Glasgow, UK
3 Queen Elizabeth University Hospital, Glasgow, UK

HealiOst is a novel system that delivers bone morphogenetic protein-2 (BMP-2) to sites of problematic bone healing. It binds BMP-2 to material surfaces by exploiting a unique interaction between the polymer poly (ethyl acrylate) and fibronectin. The system allows efficient delivery of an ultra-low dose of BMP-2, potentially improving safety compared with other systems currently in clinical use. In 2017, the first ever clinical application saw HealiOst successfully unite an infected and non-healing humeral fracture in a dog (Cheng *et al*, 2019). Subsequently we have treated a further seven dogs and two cats: three fracture non-unions, two delayed unions and one arthrodesis that failed to fuse, implant failure in a femoral fracture, a distal radial peri-implant fracture, persistent intertarsal subluxation following talocrural fusion and a radial valgus deformity caused by fracture malunion. Outcomes were initially assessed using standard clinical examination and radiography. Six of the nine cases have achieved bony union. There have been no instances of excessive bone formation, or any other significant complications caused by HealiOst. We have seen good or excellent limb function in five of the nine cases at short-term follow-up, and satisfactory function in one. One case was euthanased due to an unrelated co-morbidity, and the fracture site containing HealiOst was then examined histologically. At the time of writing (January 2020), follow-up of two cases is ongoing. We are also now seeking objective, long term follow-up and blinded scoring of the radiographs using a modified Radiographic Union Score for Tibial fractures (RUST) system.

References

Cheng ZA, Alba-Perez A, Gonzalez-Garcia C, *et al.* Nanoscale coatings for ultralow dose BMP-2-driven regeneration of critical-sized bone defects. Adv Sci 2019, 6, 1800361 **https://doi.org/10.1002/advs.201970009**

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Find a Better Way

BIOFUNCTIONAL AND ANTIBACTERIAL TITANIUM NANOTOPOGRAPHY FOR ORTHOPAEDIC IMPLANTS

Laila Damiati[1], Virginia Llopis-Hernández[1], Pefing Li[2], Richard Oreffo[3], Gordon Ramage[4], Penelope M. Tsimbouri[1], Bo Su[5]; Manuel Salmeron-Sanchez[1], Matthew J. Dalby[1]

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Orthopaedic device-related infection (ODRI) is one of the major complications in orthopaedic surgery. Infections caused by Gram-negative bacilli, including *Pseudomonas aeruginosa* account for approximately 6%– 17% of ODRI. Furthermore, as the antibiotic treatment starts to lose effectiveness new approaches become required. Such approaches can include bioactive coating and implants with nanotopography. Here, we applied these two approaches on titanium (Ti) substrates to aim to reduce bacterial attachment while enhancing osteogenesis. Ti nanostructures were produced using an alkaline hydrothermal method to produce antibacterial high aspect ratio features followed by coating with poly-ethyl acrylate (PEA) using plasma polymerisation to create a thin layer of polymer. PEA causes spontaneous revealing of fibronectin (FN) upon contact which helps to expose the cell (e.g. osteoblast) adhesion and growth factor binding domains that allow the bone morphogenic protein-2 (BMP2) to adsorb on the surface; this coating is to promote osteogenesis. The optimised surface showed a reduction in the *P. aeruginosa* attachment and change in bacterial metabolite pathways. Enhanced MSC osteogenesis with the PEA/FM/BMP2 coating on the nanotopography was confirmand using different techniques, such as Raman spectroscopy, qPCR, Calcine blue, and Giemsa staining. Furthermore, MSCs cultured on the coated nanotopography substrate in the presence of *P. aeruginosa* or with their quorum sensing molecules (C12-HSL and C4-HSL) were more resistant to infection compared to the MSCs growth on the flat surface. These aspects suggest that the developed novel nanotopography/ bioactive coating could promote bone regeneration and reduce the biofilm formation.

IN SILICO TRIALS AND MACHINE LEARNING FOR ORTHOPAEDICS AND TRAUMA

Richie Gill [1,2]

1Department of Mechanical Engineering, University of Bath, UK,
2Centre for Therapeutic Innovation, University of Bath, UK,

Abstract

This talk will cover the use of two cutting-edge computer methods for advancing orthopaedic and trauma. The first is the use of in silico trials for establishing safety equivalence for new devices. An in silico trial allows a new paradigm, cohorts under investigation can be duplicated and given treatments from both arms of a study, allowing paired statistics and eliminating bias between arms. Hip fracture diagnosis and classification is difficult, and there is a relatively low level of agreement in terms of classification amongst human observers. Machine learning offers a possibly more reliable and repeatable method for classification.

MICRORNA EXPRESSION AND FUNCTION IN FRAGILITY FRACTURES

R.K. Silverwood[1], R.M.D. Meek[2], C.C. Berry[1], M.J. Dalby[1]

1. Centre for the Cellular Microenvironment, Institute of Molecular, Cell and Systems Biology, University of Glasgow

2. Department of Trauma & Orthopaedics, Queen Elizabeth University Hospital, NHS Greater Glasgow & Clyde

Osteoporosis represents an ever increasing burden to health care services world-wide. Within the UK alone, over 500 000 fragility fractures secondary to osteoporosis occur each year, resulting in significant morbidity and mortality. Furthermore, the management of these injuries cost health care services billions of pounds. A revolution in the diagnosis and treatment of the disease is required to prevent significant suffering of future generations.

MicroRNAs are known to regulate many key physiological processes, including mesenchymal stromal cell (MSC) differentiation. They have been demonstrated to be abnormally expressed in many musculoskeletal conditions, including osteoporosis. Patients who have suffered a neck of femur fracture are known to be at risk of, or have undiagnosed, osteoporosis. Dysregulated microRNAs have been identified in bone and serum samples of patients with this injury type. Great hope has been placed on the development of targeted therapies to manipulate microRNA expression and improve bone quality.

MicroRNA expression was analysed, and key microRNAs, microRNA-143 and microRNA-31, were manipulated using functionalised gold nanoparticles to further understand their role in MSC differentiation. Furthermore, the metabolomic effect of inhibiting microRNA-31 was explored to further understand its role in osteogenesis.

These results provide further insight to the impaired MSC function of patients who have suffered a fragility fracture. Furthermore, the potential of microRNAs to be utilised as a biomarker or therapeutic target for osteoporosis has been reinforced.

NANOSCALE VIBRATION TO MODULATE OSTEOCLASTOGENESIS

Ian Kennedy [1], P. Monica Tsimbouri [1], Dominic R.M. Meek [2], Carl S. Goodyear [3], Matthew J. Dalby [1]

[1] Centre for the Cellular Microenvironment, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, Joseph Black Building, University of Glasgow, Glasgow, G12 8QQ. [2] Department of Trauma and Orthopaedics, Queen Elizabeth University Hospital, Glasgow, G54 4TF. [3] Institute of Infection, Immunity and Inflammation, Glasgow Biomedical Research Centre, University Place, Glasgow, G12 8TA.

Introduction: Mechanical factors have been shown to significantly influence stem cell differentiation and fate. Researchers have demonstrated that nanoscale vibration can promote osteogenesis in isolated mesenchymal stem cell (MSC) cultures. In the bone marrow niche, there is a co-dependent existence between MSCs and cells from the haematopoietic lineage (HSCs), particularly osteoclasts. While MSC derived osteoblasts stimulate new bone formation, osteoclasts break down bone. Given the close overlap between these two cells types, an investigation in to the effect of nanoscale vibration on osteoclasts was required.

Methods: Two culture methods were used: an isolated culture of osteoclasts and osteoclast-precursors, and a co-culture of bone marrow derived MSCs and HSCs. Vibration was produced with the nanokick bioreactor – a recently developed technology that facilitates the delivery of accurate and reproducible nanoscale vertical displacements. This bioreactor allows otherwise standard cell culture techniques to be used. A range of experiments was used to investigate the effect of nanoscale vibration, including immunostaining, resorption analysis, RT-PCR, ELISA and metabolomics.

Results: Nanoscale vibration was found to influence osteoclast differentiation and function. A reduction in osteoclast numbers was observed in both culture conditions. Furthermore, less resorption occurred in the nanokick group. There was no significant impairment in osteoblast development or function when osteoclasts were present, with evidence of increased cytoskeleton tension and mineralisation following stimulation. A number of gene, protein and metabolome changes were observed, suggesting a state of lower inflammation in the nanokick group.

Conclusion: It is hoped that these results will provide further evidence to validate the use of the nanokick bioreactor as a method of producing tissue-engineered bone graft for clinical applications.

3D modelling of the bone marrow niche as a potential platform for testing new therapeutic approaches in Chronic Myeloid Leukaemia.

Caroline Busch [1,2], Theresa Mulholland [3], Matthew J. Dalby [1], Michele Zagnoni [3], Helen Wheadon [2], Catherine C. Berry [1]

1 Centre for the Cellular Microenvironment, Institute of Molecular, Cell and Systems Biology, University of Glasgow, Glasgow, UK,
2 Paul O'Gorman Leukaemia Research Centre, Institute Cancer Sciences, University of Glasgow, Glasgow, UK
3 Department of Electronic and Electrical Engineering, University of Strathclyde, Glasgow, UK

Chronic myeloid leukaemia (CML) is characterised by the excessive proliferation of leukaemic stem cells (LSCs) in the bone marrow (BM). LSCs achieve this via modification of the BM microenvironment -the niche- to their advantage, whilst impairing normal haematopoiesis. To date it has proved difficult to both understand how LSCs dominate and alter the niche and to target LSCs with current therapies. In the healthy BM, mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSCs) reside together in the niche, where they interact closely, maintaining their stem cell properties via self-renewal. Most in vitro systems representing the niche are basic, relying on 2D-models consisting of a stromal monolayer in co-culture with HSCs. However, such systems overlook many niche factors, including the BM 3D architecture. 3D-culture systems provide a more realistic reflection of the BM microenvironment in vitro and can better predict in vivo responses of chemotherapy in disease modelling.

We are working on 3D BM niche-models, comprising MSC spheroids embedded in medical-grade collagen type I, mimicking the BM biological and mechanical microenvironment. We have extended this model to include LSCs and use our model to study MSC-LSC interactions within a niche-like environment and LSC-mediated remodelling. Initially, we investigated LSC invasion using a collagen type I coated Transwell/MSC culture, demonstrating migration into the collagen network and homing to the MSCs beneath. Subsequently, we introduced the LSCs to our BM-model and noted migration into the gel scaffold. Our artificial 3D BM model enables us to study the cell-cell interaction within a niche-like environment, and the processes the LSCs undertake in order to remodel the bone marrow to their advantage.
In parallel, LSC monoculture experiments have revealed new potential CML treatment approaches, a combination of tyrosine kinase and bone morphogenetic protein inhibitors. We observed a synergistic mode of action displaying changes in cell cycle, increase in apoptosis and fewer cell divisions. Furthermore, we investigated MSC spheroid drug sensitivity, using microfluidic devices. Microfluidic devices are a powerful tool to study MSC spheroid formation and LSC-MSC interactions, and can be used as a platform for testing new therapeutic approaches. We observed no changes in MSC spheroid growth or viability upon treatment which ensures specific targeting of leukaemic cells with our inhibitors.
Future experiments will aim to assess inhibitor effect on LSCs in the presence of MSC spheroids, firstly in a microfluidic system and secondly using our collagen model.

MICROBIOME-DERIVED CARNITINE MIMICS AS NOVEL MEDIATORS OF GUT-BRAIN AXIS COMMUNICATION

Heather Hulme[1], Lynsey M. Meikle[1], Nicole Strittmatter[2], Justin J.J. van der Hooft[3], RuAngelie Edrada-Ebel[4], Victor H. Villar[5], Saverio Tardito[5], Richard J. A. Goodwin[2], Richard Burchmore[1], Dónal (Daniel) Wall[1]

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3Bioinformatics Group, Wageningen University, The Netherlands
4Natural Products Metabolomics Group, University of Strathclyde, UK
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Alterations to the gut microbiome are associated with various human diseases, yet evidence of causality and identity of microbiome-derived compounds that mediate host-microbe communication remain elusive. Focusing on the gut-brain axis we identified two novel bacterial metabolites that are structural analogues of carnitine and are present in both gut and brain of specific pathogen free mice, while completely absent in germ free mice. We demonstrate that as well as being structurally similar to carnitine, these compounds co-localize with carnitine in brain white matter, and inhibit carnitine mediated fatty acid oxidation in a murine cell culture model of white matter. The *Lachnospiraceae* producing these metabolites are a relatively poorly understood family of bacteria, although recently their isolation in reproducibly high numbers from the human gut microbiome in specific diseases drew attention to their potential role in certain conditions. Our work is the first description of direct molecular inter-kingdom exchange between gut prokaryotes and the mammalian brain, leading to inhibition of brain cell function and we believe that there is compelling evidence for a more widespread role of these molecules in human health and disease.

CRITICAL BONE DEFECTS: OPPORTUNITIES AND OBSTACLES

David Shields[1], Virginia Llopis-Hernandez[2], Cristina Gonzalez-Garcia[3], Vineetha Jayawarna[3], Matt Dalby[2], Manuel Salmeron-Sanchez[3]

1.Department of Trauma and Orthopaedics, Queen Elizabeth University Hospital, Glasgow
2.Centre for Cell Engineering, Institute of Molecular Cell and Systems Biology, University of Glasgow
3.Division of Biomedical Engineering, University of Glasgow

Introduction. Critical bone defects, are loss of bone which will not spontaneously heal, due to the amount of bone loss. They occur clinically most commonly as a result of trauma, infection, tumour resection or non-union. The present strategies available to clinicians are limited by either unpredictable graft performance or unacceptable functional/symptomatic consequences for patients. This work outlines the need for an engineered bone inducting (osteoinductive) graft and demonstrates some of the limitations to its implementation in clinical practice. The osteoinductive system explored in particular is a polymer-protein nanocoating (poly ethylacrylate & fibronectin) able of presenting growth factors, in particular bone morphogenetic protein 2 (BMP-2), in ultra-low doses.

Methods. Resilience of the nanocoating to ethylene oxide (EO) was carried out by comparison of EO sterilised samples to freshly prepared controls for topography (atomic force microscopy), functionality (ELISA) and biocompatibility (C2C12 differentiation). The use of canine bone chips as a carrier scaffold for the nanocoating was evaluated in a murine *in vivo* critical defect model. Finally, the prospects of a fully synthetic 3D printed scaffold (poly caprolactone) was explored for mechanical and biological compatibility using compression testing *in vitro* cell culture.

Results. Following EO sterilisation, the topographic appearances of the nanocoating remained consistent with their non-sterilised counterparts. Functionality remained the same for the protein coating, however the addition of BMP-2 was not found to resist the 1 week long sterilisation process. Cells were able to differentiate at a statistically similar rate whether materials were sterilised or not. The use of bone chips as carrier graft for the nanocoating was found to subjectively promote mature bone formation histologically, however quantitative analysis was confounded by the persistent present of mineralised bone chips. Fully synthetic scaffolds were mechanically inferior to currently used load bearing materials. The reduction of porosity was associated with improved mechanical properties, however not to a required load bearing degree. The addition of HA into the PCL matrix did not improve mechanical properties, however all scaffolds remained capable of inducing bone formation using mesenchymal stem cell cultures.

Conclusions. The use of this polymer-protein nanocoating to present BMP-2 is topographically and functionally resilient to sterilisation and demonstrates early translational promise. The optimal scaffold carrier is yet to be established and further work on industrial limitations is required.

ESTABLISHING A UNIQUE 3D CO-CULTURE SYSTEM TO MODEL AND THERAPEUTICALLY TARGET LEUKAEMIA-BONE MARROW MICROENVIRONMENT INTERACTIONS IN ACUTE MYELOID LEUKAEMIA.

Aikaterini Miari[1], Helen Wheadon[2], Monica Guzman[3], Mark Williams[1]

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Background: Clinical outcomes for the majority of Acute Myeloid Leukaemia (AML) patients remain sub-optimal. Chemoresistance mediated by cross-talk between the leukeamic cells and the bone marrow microenvironment (BMME), is a key factor responsible for inferior survival in AML [1]. An enhanced understanding of leukemic cell–BMME interactions will drive novel drug development and/or instruct drug repositioning to enhance AML patient survival. **Rationale:** Approaches for modelling and therapeutically targeting leukaemia–BMME cross-talk (i.e. 2D co-culture models) fail to recapitulate key features of the BMME, including: hypoxia; leukaemic cell clustering and the complex interplay between AML cells and BM-resident cells, including fibroblasts and macrophages. This has led to 2D culture systems significantly overestimating drug efficacy compared to 3D model systems. As a result, agents identified from studies using existing 2D *in vitro* model systems, have yielded suboptimal clinical outcomes in the treatment of resistant/relapsed AML [2]. **Aim:** To establish a unique *in vitro* 3D (spheroid) co-culture system that originally reflects internal conditions within the BMME. **Methods:** U937 and THP-1 ($CD45^+$) AML cell lines were cultured alone or co-cultured with a human fibroblast cell line expressing green fluorescent protein (HS5-GFP[$CD45^-$]) or primary human monocyte derived macrophages in agarose-coated or ultra-low adherent (ULA) 96 well round bottom plates. Spheroid formation, leukaemic cell clustering and establishment of hypoxic conditions were assessed via the EVOS Cell Imaging System together with the Image-iT Green Hypoxia Reagent. Leukaemic cell separation from GFP^+ stromal counterparts were undertaken using CD45 magnetic activated cell sorting (MACS), with AML cell purity determined by the MACSQuant Analyzer 10 flow cytometry, based on differential GFP expression. **Results:** HS5-GFP^+ cells formed compact and defined spheroids, with primary macrophages forming clusters within 24 hours of cultivation. U937 and THP-1 cells formed clusters in monocultures, and around the HS5-GFP^+ cells and primary macrophages in the co-cultures within 24 hours. Hypoxic conditions were detected within the U937 and THP-1 clusters and in the stromal core of the co-cultures within 24 hours post culture. Leukaemic cell purities of ~95% were achieved with U937 cells after 24 hours of co-culture with HS5-GFP^+ cells, via CD45 MACS. **Discussion/conclusion:** This system will now be used to investigate targetable chemoresistant mechanisms in AML.

[1] Burnett, A.K. Hematology Am Soc Hematol EducProgram 2012, 1–6 (2012).
[2] Lapusan, S. Invest New Drugs 30, 1121–1131 (2012).

MAKING CONNECTIONS: USING ANATOMY TO GUIDE TISSUE-ENGINEERED DESIGN

Jennifer Z Paxton

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Tissue engineering is an emerging field of research focussed on the manufacture of body tissues and organs in the laboratory. It combines the basic principles of biology with engineering and materials science to produce tissue-like structures with the potential for implantation. While research within tissue engineering is making significant advances, a major drawback regarding the implantation of tissue-engineered structures remains the creation of suitable tissue interfaces. This is extremely relevant within the musculoskeletal field, where the main function of the tissue is to generate or transmit force. Without suitable mechanical and biochemically graded transitions between the tissues of muscle, tendon, ligament and bone, stress and strain concentrations would cause tissue injury and in the case of an implanted tissue, certain implant failure. For example, clinically, bone to tendon/ligament healing results in a weak interface, formed mainly of scar tissue, which does not possess the graded structure present in native tissue. This means that, even if advances in biomaterials research and tissue engineering reach the point of creating tissue analogues possessing all the desired native tissue characteristics, adequate fixation and interface generation on implantation will always remain the biggest challenge for a successful clinical outcome.

This talk will describe the current progress in engineering tissue interfaces in the laboratory and how we use anatomy to guide the design and development of structurally relevant models and implants. It will also examine the methods currently under investigation to achieve the ultimate goal of functional tissue replacement.

NANOPARTICLE LABELLING OF THERAPEUTIC CELLS

GC Higgins,[1,2] SE Thomson,[1,2] M Kearns,[1,2] E Ross,[1] C Berry,[1] JF Riddell,[1] R Wallace,[2] MO Riehle,[1] AM Hart.[1,2]

University of Glasgow,[1] Canniesburn Plastic Surgery Unit, Glasgow Royal Infirmary,[2] University of Edinburgh.[3]

Introduction: Peripheral nerve injury is common and in an effort to improve outcomes alternatives to nerve grafting are sought. The literature suggests human Adipose Derived Stem Cells (ADSC) and ADSC differentiated to Schwann cell phenotype (dADSC) may improve nerve regeneration, however, a lack of *in vivo* cell tracking and use of animal derivatives in cell culture pose barriers to clinical translation. This study examines Super Paramagnetic Iron Oxide Nanoparticle (SPION) labelling of therapeutic cells and compares traditional animal serum culture medium supplement to human Platelet Lysate (hPL).
Materials and Methods: The ADSC were extracted from human adipose, as per Human Tissue Act (2004) and Biobank approval (314). Cells were cultured in medium supplemented with Foetal Bovine Serum (FBS) or hPL and an established differentiation protocol (Kingham et al. 2007) was implemented. Cells were labelled with fluorescent, 200nm SPIONs at 0.01mg/ml and 0.1mg/ml concentration. ADSC stemness was assessed by flow cytometry, viability by Live Dead assay, proliferation by growth curves, and phenotype by morphology and immunohistochemistry analysis.
Results: ADSC retained markers of stemness (CD90, CD73 and CD105) irrespective of medium supplement and SPION labelling. 98% and 96.4% of ADSC and dADSC exhibited uptake of nanoparticles, respectively. Cell viability was SPION dose dependent and was better for cells cultured in FBS compared to hPL supplemented medium ($p=<0.05$). hPL and dADSC culture media and low concentration SPION labelling were associated with increased cell proliferation and induced a more spindle shaped morphology ($p<0.0001$). hPL supplemented media in combination with high SPION concentration reduced cell viability (79%). dADSC stained positively for Schwann cell markers s100/GFAP in the absence and presence of SPIONs.
Conclusion: These results suggest that low concentration SPION labelling and hPL supplemented media have potential for use in the development of stem cell therapy for peripheral nerve injuries.

MECHANISTIC AND METABOLIC INSIGHTS INTO BIOENGINEERING THE BONE MARROW NICHE *IN VITRO*

Hannah Donnelly[1], Ewan Ross[1], Christopher West[2], Bruno Peault[2], Manuel Salmeron-Sanchez[1] & Matthew J Dalby[1]

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Stem cells lose their regenerative capacity when they are removed from their regulatory microenvironment, termed the stem cell niche. Perivascular stem cells, or pericytes, are key bone marrow niche cells, they act to support haematopoietic stem cells (HSCs) in the bone marrow (BM). However, upon ex vivo culture self-renewal their capacity is lost. In order to maintain 'stemness' in culture, aspects of the BM microenvironment should be recapitulated in vitro. Here, we aim to create a system supporting a niche-like pericyte phenotype, investigate mechanisms important for self-renewal and assess HSC maintenance. Noting that soft gels can support nestin[1] expression, a key niche marker, we have shown stiffness related support of other key markers important for a niche-like phenotype, including surface markers and HSC maintenance cytokine expression. Here, poly(ethyl acrylate) (PEA) was used to assemble fibronectin (FN) into physiological-like networks, thus allowing growth factor tethering and presentation in synergy with integrin binding sites[2]. Then, a low-stiffness gel or hypoxia was used to investigate the metabolic mechanisms required to support these phenotypes in this niche-like microenvironment. Transcriptomic analysis and flow cytometry identified changes in key niche and differentiation related genes supported by the different systems, such as increased expression of nestin when the low-stiffness gel is added. Addition of low-stiffness gels also revealed an increase of sustained activate HIF1α (hypoxia-inducible factor 1α) compared to hypoxic conditions and controls. Metabolomic screening highlighted strong agreement in down-regulation of metabolites involved in oxidative phosphorylation with the low-stiffness gel and hypoxic system, pointing to maintenance of a hypoxic-like metabolism suggestive of a quiescent HSC-regulatory phenotype. However, downstream analysis of HIF1a-driven VEGF production and lactate dehydrogenase levels did not increase with gels, suggesting differing mechanisms of action. Upon co-culture with HSCs significant maintenance of long-term repopulating HSCs was observed only when in the presence of low-stiffness gels.

We have demonstrated the PEA system can be utilised to probe aspects of the BM niche microenvironment, allowing investigation into fundamental mechanisms important in stem cell regulation. The addition of low-stiffness gels leads to a 'hypoxic-like' mechanistic response that could be important in maintaining this HSC-supportive phenotype. This system demonstrated that low-stiffness was required to maintain the most naïve and clinically useful HSC population. Systems such as these can have large implications for ultimately the production of clinically relevant HSCs in the clinic, but also provide a platform on which to investigate important niche mechanisms in both stromal cells and HSCs.

[1]Engler AJ, Sen S, Sweeney HL, et al. Cell 2006; 126: 677-89.
[2]Llopis-Hernandez V, Cantini M, Gonzalez-Garcia C, et al. Sci Adv 2016; 1-11.

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Sponsor Presentations

NEAR INFRARED DETECTION TECHNOLOGY – A ROBUST SOLUTION FOR QUANTITATIVE BIOASSAY IMAGING

Shula Dawson[1]

1 Biotechnology Scientific Solutions and Support Group, LI-COR Biosciences Ltd, Cambridge, UK

Near Infrared (NIR) fluorophores provide exceptional sensitivity and high signal-to-noise ratios for detection and quantification in a region where light absorption and autofluorescence of cellular and tissue components is low. Imaging agents, cells, antibodies and molecules labelled with these fluorophores allow biochemical activity measurements of *in vitro* and *in vivo* assays with high sensitivity and reproducibility. Using high resolution imaging and scanning technology in the NIR region of the spectrum allows a fuller understanding of the details of protein expression, ligands, molecular targets, pathways, and cellular effects across a range of different bioassays including membrane, gel, cell, and tissue formats, as well as small animal *in vivo* applications. This allows an integrated approach to pre-clinical investigations spanning multiple stages from initial characterisation of biological markers, through functional studies and therapeutic development all the way to clinical translation.

Biocomposites presentation

TREATMENT OF INFECTION WITH STIMULAN

Bethany Loughlin

PromoCell presentation

Andrew Woods

Poster abstracts

1. ENGINEERED BACTERIAL BIOFILMS TO CONTROL STEM CELL DIFFERENTIATION

Aleixandre Rodrigo-Navarro [1], Jake Hay[1], Michaela Petaroudi[1], Matt Dalby [2], Manuel Salmeron-Sanchez [1,2]

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Introduction. The use of materials for stem cell culture is attractive since it can be easily upscaled using techniques such as 3D printing or fibrous hydrogel scaffolds. These materials can be engineered to deliver specific and/or dynamic cues to the stem cells. However, these systems lack the ability to deliver the precise cues required for stem cell differentiation in a controlled manner, since stem cells usually reside in a highly complex niche and their fate is tightly regulated by a plethora of factors.

We present a novel approach using bacteria as a substrate to influence mesenchymal stem cells in a facile and temporal manner. *Lactococcus lactis* spontaneously develops biofilms on a variety of surfaces (e.g polymers, metals) and can be genetically modified to produce a variety of probiotic and efficacious proteins[1,2]. Here we show that controlled expression of fibronectin fragments supports growth and temporal regulation of secreted bone morphogenetic protein 2 drives osteogenesis in an on-demand manner.

Methods. *Lactococus lactis* NZ9020 has been genetically modified to express the III7-10 fragment of human fibronectin and human bone morphogenetic protein 2 (BMP-2) in an inducible fashion. Protein expression was characterised with ELISA and Western blot.

Human MSCs were co-cultured with *L. lactis* biofilms for up to 28 days and assessed for osteogenic differentiation using von Kossa for mineralization, osteocalcin immunofluorescence and ALP expression.

Results and discussion. *Lactococcus lactis* develops stable biofilms on a variety of substrates. These biofilms contain viable bacteria and are stable for up to 28 days, supporting hMSC adhesion, proliferation and differentiation.

Lactococcus lactis expresses biologically active III7-10 fibronectin and BMP-2. The expression levels of BMP-2 can be adjusted and induce osteogenic differentiation, as assessed with the techniques described in the previous section. The behaviour of hMSCs cultured on engineered *L. lactis* biofilms is equivalent to the use of a fibronectin coated substrate with the addition of 100 ng/mL of BMP-2, the standard for osteogenic differentiation of hMSCs.

Conclusion

Engineered *L. lactis* biofilms expressing FN III7-10 and BMP-2 in an inducible manner can be as a functional, living biointerface between a wide range of substrates and hMSCs to drive its differentiation to the osteogenic lineage in a predictable and controlled way.

References

1. Rodrigo-Navarro, A., Rico, P. Scientific Reports, 4, 5849 (2014)
2. Hay, J. J., Rodrigo-Navarro, A. Scientific Reports, 6, 21809 (2016)

2. DEVELOPMENT OF A MULTIPURPOSE ALG/GEL BASED PLATFORM FOR 3D BIOPRINTING AND ITS APPLICATION FOR BONE ENGINEERING

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Introduction. 3D Bioprinting has arisen as a key enabling technology which by combining biomaterials, cells and biomolecules is capable of fabricating structures that emulate tissues and organs present in the human body. This summed up to the fact that it allows the possibility of tailoring the fabricated constructs, via. patient based geometries or specific cell lading, represents a paradigm shift in medicine, allowing for extensive personalized medicine approaches. In this work we present a 3D Bioprinting platform, including a set of designs, workflows and a family of alginate-gelatine based bioinks that could be used for many different end applications due to granting control over their physicochemical properties and the possibility of further tuning with biomolecules tailored for specific tissue types. We also used the proposed platform for bone engineering with the creation of vascularized bone disks that could either be used as bone in vitro models or stacked to be used as bone defect fillers implants.

Methods. The family of Alg/Gel-based bioinks was developed by dissolving the given polymers at different ratios in various solvents (MiliQ water, PBS and cell culture media). The printed Alg/Gel structures were exposed to different concentrations of $CaCl_2$ solutions for different times to crosslink after printing.

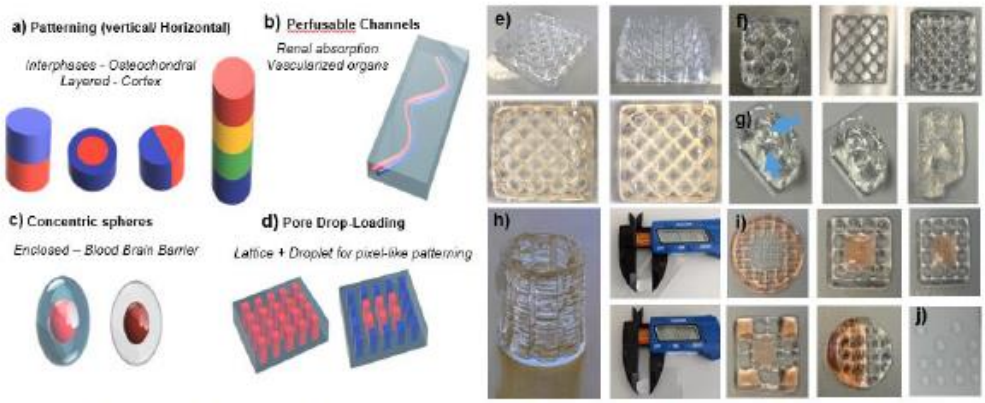

Figure.a-d)Diagram showing different multimaterial designs and its possible applications. e)2cm lattice structures. f)1cm lattice structures with 3,2.5,2mm infill spacing. g)2cm Human ear construct. h) Collapsible hollow tube. j)1cm Multiple material constructs. j)Droplet based microgels

HTERT cells previously expanded in regular cell culture conditions, were laden into the hydrogels and then loaded into printing cartridges. The cartridges were then placed in the printheads and layered into different pre-designed structures by using a commercially available bioprinter from RegenHu. Cellular viability was assessed using Live/ Dead assays. Several printed structures were designed having various end applications in mind. Some of these included multiple materials, belonging to the Alg/Gel family but also Gelatine-Methylacryloyl (GelMA)

photocrosslinkable bioinks, which allowed for the creation of perfussable channels, by incorporating sacrificial geometries.
Results. Rheology data showed control over the mechanical properties amongst different Alg/Gel bioinks, being able to obtain materials with Young's Modulus ranging from 4 KPa to more than 40 KPa. Cellular viability inside the printed constructs was also found to be over 90% after printing and even distributions of the cells throughout the construct was ensured.
The proposed printing platform was used to print different structures spanning from standard lattices, commonly used for bone engineering to human-size ears that could represent future implants for cartilaginous body parts, collapsible hollow tubes that could be used for vessel modelling in vitro and high-throughput droplet-based microgels. Printed constructs were composed by up to 80 layers, sizes varying from 1 cm to 4 cm depending on the construct.
Additionally, vascularized chips were designed and fabricated, which included multiple materials, a main GelMA hydrogel enclosing a sacrificed Alg/Gel structure. These gel chips have an inner channel network enclosed in a solid structure, serving potentially as co-culture models for in vitro applications, allowing the perfusion of solutions that will better mimick and ensure appropriate nutrient distribution within the main structure.
Multimaterial approaches were also used to design and print 7 mm disks with differentiated areas. Congo red was use to visually assess the separation, while NeutrAvidin's conjugates were used to fluorescently tag the different areas and estimate protein diffusion within the structure. Structures with different stiffnesses distributions were also obtained via this method.
Conclusions. The developed platform showed great versatility, firstly due to the tunability of the bioink's mechanical properties facilitating the utilization of a variety of cell types, as well as per the different structures obtained using multiple printing approaches. Potentially, this platform could be employed for the creation of complex in vitro models used to study biological processes, systems or diseases, which could also include enhanced biomimicry, e.g. incorporating inner channels. Specific cell types obtained from patients could be added to these models to assess drug toxicity or efficacy, highlighting the platform's potential in the development of future personalized medicine approaches.

3. SEEKING THE LINK BETWEEN MECHANICAL AND METABOLIC ACTIVITIES IN CELL DIFFERENTIATION

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INTRODUCTION. Cells sense the environment stablishing focal adhesions (FAs) formed by the attachment of
integrins with proteins from the extracellular matrix (ECM) (e.g. fibronectin (FN)). Every information gathered by cells from these links will play an important role in their fate [1]. Therefore, it is important to understand cell-ECM interactions and what intracellular changes occur upon these interactions. We hypothesise that to create an adaptive response to ECM signals, cells exert forces in response to the surrounding matrix, and this requires a mechanical energy that will have an implication in the biology of the cell [2]. This work aims to understand how cells test the environment from a mechanobiological perspective during their attachment, proliferation, migration and differentiation. This will provide a better understanding on how microenvironmental cues can be used to control cell fate, enhancing studies in multiple fields e.g. tissue regeneration or drug testing.

METHODS. The proposed system consists on a 2D Full-length Fibronectin- based PEG hydrogels model developed in our group [3]. This system allows stiffness control by varying the amount of PEG and also, control over the degradability of the matrix by adding protease-sensitive crosslinkers. Murine L929 fibroblasts were used for optimisation of the system. Forces exerted by cells were analysed using traction force microscopy (TFM). In order to do this, fluorescent microspheres were embedded in the gels. Stacks of the position of the beads after and before cells trypsinisation were analysed to calculate displacements, which yields gel's stress and hence, the forces exerted. Also, a myosin II inhibitor (blebbistatin), was added to the media to study the implication of cytoskeletal tension in force generation.

RESULTS AND DISCUSSION. Cells pull less on non-degradable hydrogels compare with degradable ones when maintaining same bulk stiffness. As expected, the inhibition of myosin II resulted in a decrease in the forces exerted by cells in both conditions. However, forces were still higher in the degradable condition compared to the non-degradable one. This could mean that the degradability of the system is an important parameter in cell mechanosensing, allowing cells to apply higher forces. Moreover, cells tend to acquire a more spread shape when blebbistatin is added, which could be related to the lack of cytoskeletal tension. In the future, all these results will be compared with the metabolic activity of the cell to understand the implication of the mechanical forces in the biology of the cell.

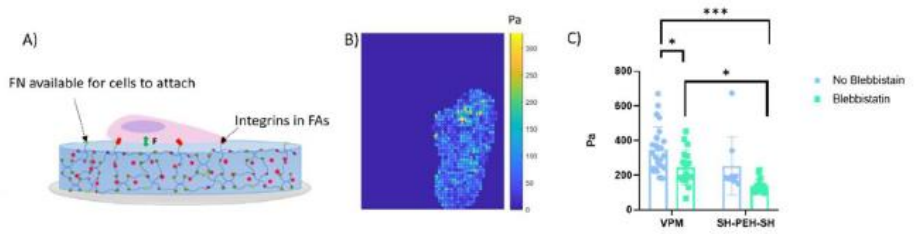

Figure 1. A) *Sketch of proposed system* ***B)*** *Traction force map of a murine L929 fibroblast.* ***C)*** *Forces applied by cells on gels with different crosslinker (VPM degradable, SG-PEG-SH non-degradable) control cells (blue, No blebbistatin) and myosin II blocked (green, blebbistatin).*

CONCLUSIONS. From these early experiments we can conclude that cells respond differently with changes in matrix stiffness and degradability. A direct evidence of how cells respond to these stimuli is through morphological changes, which indicates that further adaptive response might be taking place inside the cell. Furthermore, these results show the great importance of the actin cytoskeleton contraction in force loading. Changes in the biophysical properties of the matrix alter how cells sense their surroundings and might have an implication in their metabolic activity, determining cell fate. Consequently, cell metabolic activity will be studied in detail in further experiments.

REFERENCES

[1] A. J. Engler, S. Sen, H. L. Sweeney, and D. E. Discher, “Matrix Elasticity Directs Stem Cell Lineage Specification,” Cell, vol. 126, no. 4, pp. 677–689, Aug. 2006.
[2] A. Elosegui-Artola et al., “Rigidity sensing and adaptation through regulation of integrin types,” Nat. Mater., vol. 13, no. 6, pp. 631–637, Jun. 2014.
[3] S. Trujillo et al., “Engineered full-length Fibronectin-based hydrogels sequester and present growth factors to promote regenerative responses in vitro and in vivo,” bioRxiv.

ACKNOWLEDGMENTS

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4. AN *IN-VITRO* ASSESSMENT OF THE EXTENDED KILLING OF BIOFILMS BEYOND THE SPACER BY ANTIBIOTIC-LOADED CALCIUM SULFATE BEADS

Craig Delury [1], Devendra H Dusane [2], Casey W Peters [2], Sean Aiken [1], Phillip Laycock [1], Ed McPherson [3], Anne Sullivan [2], Jeffrey Granger [2], Paul Stoodley [2]

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3UCLA, California, USA

Introduction: Antibiotic-loaded spacers and absorbable beads are used in the management of orthopaedic infection to provide prolonged high local concentrations of antibiotics required to better kill bacterial biofilms. In quiescent areas of the joint space, diffusion will control the spread of antibiotics; therefore, the distribution of beads may be important to ensure adequate antibiotic coverage. Do antibiotic-loaded absorbable calcium sulfate beads (ALCSB*) provide a greater zone of coverage and killing of biofilms than antibiotic-loaded PMMA spacers alone?

Method: Biofilms of bioluminescent strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were formed on stainless steel, hydroxyapatite, titanium and polyethylene coupons for 3 days. Diffusion experiments were performed in large glass plates as (i) control PMMA spacer with no antibiotics, (ii) a PMMA spacer with vancomycin and tobramycin (2000 mg of each per 40 g pack) and (iii) an antibiotic loaded PMMA spacer plus ALCSB containing vancomycin and tobramycin (1000 mg and 240 mg per 10cc pack respectively). The spacer was placed in the centre of the glass plate and the coupons placed radiating from the spacer. The ALCSB were spread evenly and the whole plate was overlaid with agar. The plates were incubated and killing of biofilms was analysed using luminescence and white light imaging.

Results: Growth and spread of *in-vitro* biofilms from the coupons was observed for the control unloaded spacer. The antibiotic-loaded spacer showed a localized zone of inhibition radiating a few mm from the spacer. The addition of ALCSB beads demonstrated a much greater zone of clearance, killing *in-vitro* biofilms on the coupons and preventing bacterial spread from the coupons.

Conclusions: In quiescent areas where the spread of antibiotics is dominated by diffusion, the distribution from an antibiotic -loaded spacer alone might be limited. The addition of Stimulan Rapid Cure beads (ALCSB) in addition to a loaded PMMA spacer can increase the area of coverage. To achieve the antibiotic concentration and exposure times required to kill or reduce biofilm bacteria, placing other reservoirs such as ALCSB may be necessary to cover as much exposed joint space as possible. Further work is required to confirm this clinically.

This study was funded by Biocomposites Ltd.

*Stimulan Rapid Cure, Biocomposites Ltd.

5. ERADICATION OF PRE-FORMED BIOFIMS USING AN ANTIBIOTIC-LOADED CALCIUM SULFATE: AN *IN-VITRO* STUDY

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Introduction: Formation of biofilms following periprosthetic joint surgery is a severe complication due to difficulties with diagnosis and eradication. Treatment for periprosthetic joint infection (PJI) often includes procedures such as surgical intervention and antibiotic therapy, whereby the most effective antimicrobial treatments are administered both locally to the infection and systemically. There is also evidence that releasing antibiotics directly at surgical sites can be used effectively in preventing implant colonisation and eradicating established biofilms. This investigation assesses the ability of synthetic recrystallised calcium sulfate (SRCS*) mixed to contain a combination of vancomycin and gentamicin (VG), or vancomycin and tobramycin (VT) to eradicate pre-formed biofilms *in vitro*.

Method: Biofilms of *Pseudomonas aeruginosa* (NCIMB 10434) or *Staphylococcus aureus* (NCTC 8325) were established on polycarbonate coupons within a CDC biofilm reactor. Biofilm coupons were exposed to plates containing suspended SRCS with VG/VT beads at concentrations of 500mg/240mg per 10cc and 1g/240mg per 10cc respectively. Control coupons were tested concurrently. Challenge plates were incubated for 24 hours at 37°C ± 2°C. All testing was performed in triplicate. Data was analysed by Students T-Test to determine statistical significance

Results: An average of 6.78 ± 0.23 $Log_{10}CFUmL^{-1}$ and of 6.60 ± 0.23 $Log_{10}CFUmL^{-1}$ of *Pseudomonas aeruginosa and Staphylococcus aureus* were recovered from the negative control biofilms respectively. No viable organisms were recovered from biofilms exposed to the positive control or those exposed to SRCS beads containing VG or VT, within the detectable limits. This equated to an average log reduction in *P. aeruginosa* of >5.78 $Log_{10}CFUmL^{-1}$ and >5.60 $Log_{10}CFUmL^{-1}$ in *S. aureus* ($p < 0.001$).

Conclusions: Exposure of *P.aeruginosa* and *S.aureus* biofilms to SRCS containing a mixture of vancomycin and gentamicin or vancomycin and tobramycin resulted in eradication of pre-formed biofilms in the method described. This may have uses in management of biofilm infections, however further assessments are required to confirm clinical performance.

This study was funded by Biocomposites Ltd.

*Stimulan Rapid Cure, Biocomposites Ltd.

6. ENGINEERING LIGAND MOBILITY IN THE ADHESIVE CROSSTALK TO CONTROL STEM CELL DIFFERENTIATION

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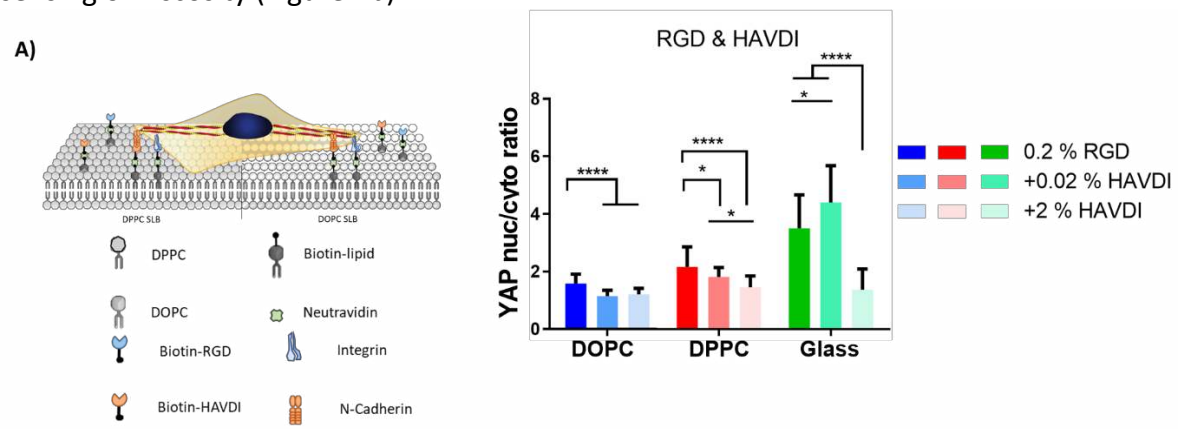
Introduction. Cell behaviour is influenced by biochemical cues mediated by interactions with the extracellular matrix (ECM) through integrins, and by interactions between cells via cadherins. On the other hand, the tissues in which the cells live are dynamic being the cells equally sensitive to the physical properties of the environment, such as stifness or viscosity, which has been traditionally ignored. In our group, we demonstrated that cells sense this property using the same mechanotransductive mechanism as with stiffness (1).
In this work, we address the role of ligand mobility (viscosity) in cell fate, and how it affects the adhesive crosstalk between integrins (RGD receptors) and cadherins (HAVDI-containing proteins) to elucidate stem cell mechanosensing of viscosity. To do this, we use supported lipid bilayers (SLBs) with varying mobility, functionalised with RGD and HAVDI peptides (Figure 1a).
Materials and Methods. Supported lipid bilayers with varying viscosities were prepared by following the vesicle fusion method. To determine how this property affects human mesenchymal stem cells (hMSCs) behaviour, two kind of bilayers were used: one of them made of DOPC, which present a fluid phase at cell culture conditions, and the other one made of DPPC, which is a gel phase bilayer. Also, glass was used as a non-mobile control surface. All the surfaces were functionalised with different ratios of RGD and HAVDI. hMSCs are then seeded on these surfaces and parameters such as cell adhesion, protein translocation or expression of transcription factors are investigated via AFM immunostraining and in-cell western.
Results and Discussion. An increase in cell area and cell adhesion, in particular its strength, is observed when the viscosity of the surface and the concentration of RGD increase. On the other hand, when HAVDI is included in the bilayers, cell spreading is reduced and changes in the location of mechanosensitive proteins (i.e. YAP) are observed, showing an altered sensing of viscosity (Figure 1b).



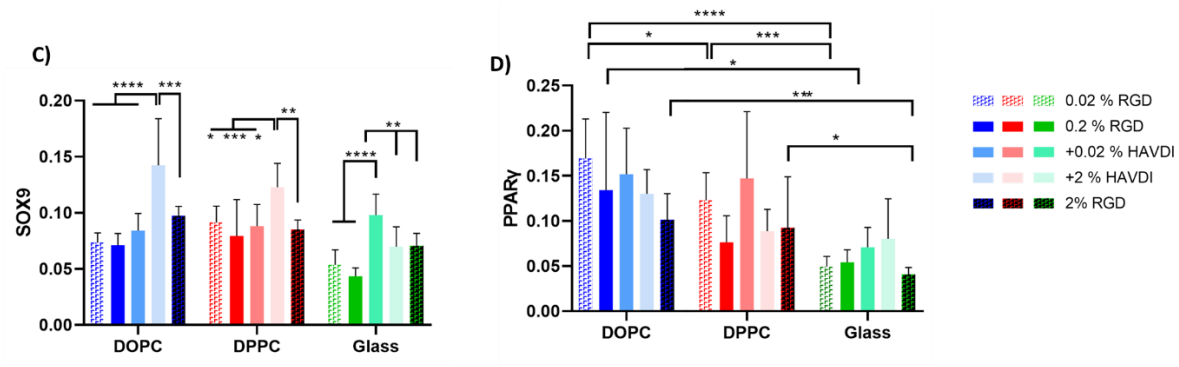


Figure 1: A) Scheme of the cells seeded on SLBs. B) YAP/TAZ ratio of h MSCs cultured on surfaces with different viscosities and amounts of HAVDI. C and D) ICW showing the expression of early differentiation markers (SOX9 for chondrogenesis and PPARY for adipogenesis) of hMSCs seeded on surfaces with different viscosities and ligands.

These changes in viscosity and ligand concentration provoke not only differences in adhesion and mechanosensing, but also in the expression of nuclear lamina and early differentiation markers (Figure 1c and d), indicating the influence of viscosity in cell fate.
Conclusions. These findings reveal the influence of viscosity in the adhesive crosstalk between integrins and cadherins as well as how this mobility affects stem cell differentiation. Our results show that when cell-cell interactions are involved, hMSCs have a different perception of the mechanical properties of their surroundings compared to when there are only ECM-cell interactions. All these changes in the sensing of the environment (viscosity, in this case) provoke changes in cell behaviour and cell fate. Further investigations on these changes will allow to finally establish a paradigm to understand and exploit cell response to viscous interactions.

References

1. Bennett M. *et al.* P. Natl. Acad. Sci. USA. *115*(6), 1192-1197, 2018

Acknowledgements

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7. Microfluidic-generated collagen particles for bone repair

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INTRODUCTION: During recent years, microgels have emerged as an effective type of drug delivery system (DDS), showing advantages such as tuneable size, increased surface area and injectability (1). Collagen is a major protein of the connective tissue in mammals, and has been extensively studied as a scaffold for regenerative medicine and drug delivery applications due to its biocompatibility, non-immunogenicity and biodegradability (2,3). The current study focuses on the adaptation of two scalable technologies, microfluidics and bioprinting, for the automated generation of monodisperse type-I collagen microgels crosslinked with multiple-arm PEG, encapsulating spheres with BMP-2 for bone regeneration.

EXPERIMENTAL METHODS: Type-I collagen (Collagen Solutions, UK) was crosslinked with 4S-Star-PEG succinimide glutarate (Jenkem, USA) to form microgels in a PDMS microfluidic device with flow focusing configuration; and in a 3D Discovery® commercial 3D-printer by successive deposition of collagen and crosslinker solution on a hydrophobic Teflon® surface. Additionally, liposomes are fabricated by membrane-extrusion and rehydration of a lipid mixture of 1,2-dimyristoyl-sn-glycero-3-phosphocholine and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DMPC and DPPC respectively). Microgels were characterized with rheological measurements, TNBSA assay and degradation studies, together with their cytotoxicity by LIVE-DEAD® using Neu-7 cells (n=3). To prove their applicability in bone regeneration, bone morphogenic protein-2 (BMP-2) was encapsulated in the microgels using the microfluidic device, and release from the biomaterial characterized using DYLight®-labelled BMP-2 and ELISA. Different osteogenic markers were evaluated (RunX2, Osteocalcin, ALP) at different timepoints *in vitro* with human mesenchymal stem cells (hMSCs). To further increase sustained release of the growth factor, BMP-2 was incorporated in the liquid phase during lipid cake rehydration and purified on a liquid chromatography column.

RESULTS & DISCUSSION: Microgels with different size and stiffness were successfully synthetized in a PDMS microfluidic device and by 3D-printing. Microgel size and mechanical properties were tuned varying the inlet channel/syringe diameter, flow speeds/pressure, pH and concentration of collagen and crosslinker. Particle size ranges from 50 to 200 µm, whereas in 3D printing the minimum size obtained was 500 µm. The comparative production rate of particles with the studied platforms, is around 600 particles/min in microfluidics, whereas in 3D printing is 30 particles/min. The microgels are non-cytotoxic to cells and foster cell growth at different crosslinker concentrations. The stiffness of the hydrogels is modulated using 4 or 8-Star shaped PEG crosslinker together with the concentration of collagen, giving rise to microgel batches that can be used for soft or hard tissue applications (figure 1, B). For bone regeneration, we tested the formulations with greater stiffness to test the differentiation of hMSCs into osteocytes. BMP-2 was released from the microgels in one week. The therefore hypothesize that the microgels serve as substrate for the proliferation of cells and contribute to their differentiation into bone-specific lineages.

CONCLUSIONS: We demonstrate that microfluidics and 3D-printing are adequate techniques for automatically generating monodisperse collagen microgels and provide a reliable tool for the posterior encapsulation of nanospheres and cells. The combination of microgels encapsulating nanometer-sized liposomes provides a controlled and sustained delivery system of BMP-2 that can be used for bone repair.

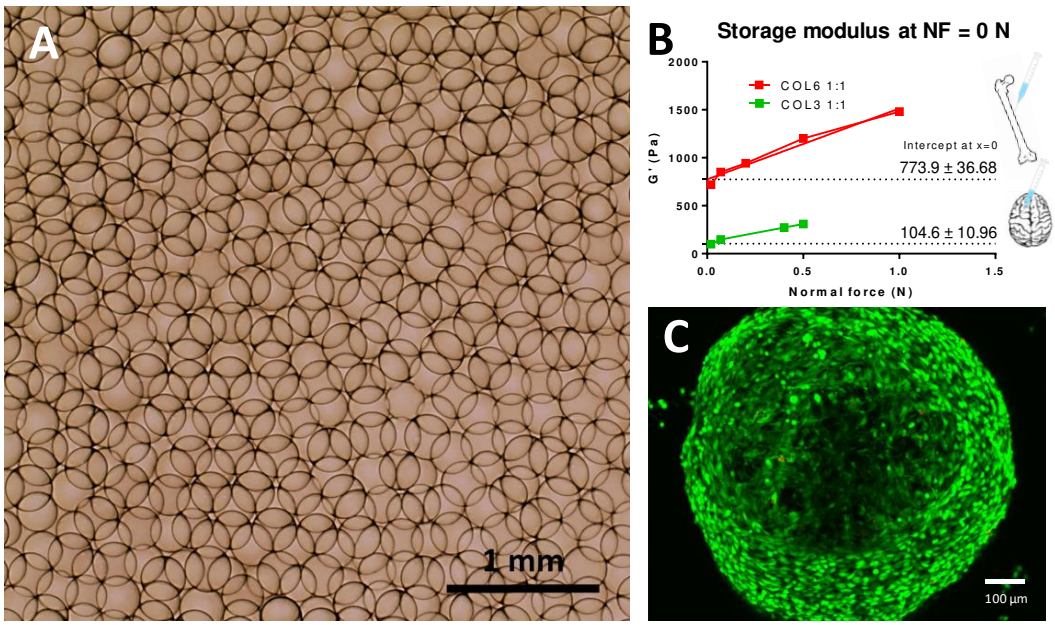

Figure 1: A. Gelification of collagen particles after synthesis in microfluidic device; B. Rheological properties of two collagen/4-Star PEG formulations C. LIVE-DEAD assay of collagen microgels; Synthesis of hydrogels in microfluidic device

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REFERENCES

[1] Oh JK, et al. 2008;33(4):448–77.
[2] Lee CH, Singla A, Lee Y. Biomedical applications of collagen. Int J Pharm. 2001 Jun19;221(1):1–22.
[3] An B, Lin Y-S, Brodsky B. Collagen interactions: Drug design and delivery. Extracell Matrix ECM ECM- Mater Ther Tools Targets Cancer Treat. 2016 Feb 1;97:69–84.

8. MICROFLUIDIC FABRICATION OF MULTILAYERED CELL-LADEN MICROGEL

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Cells within the extracellular matrix (ECM) of most tissues are orderly grouped based on cell type and lineage. To recreate analogous tissues in vitro, most cocultured models rely on embedded cells to reorganise themselves into layers. Such methods often result in a time-consuming low yield of coculture models each with a different population density ratio among two or more cell types. This difference, in turn, ensues in non-identical colocalisation of cells. Herein, we report a droplet-based microfluidic platform that can be utilised for producing miniaturized layered hydrogel laden with different cells types. The Lab-on-a-Chip nature of this method provides for a high degree of monodispersity and encapsulation efficiency in a high-throughput manner. In this report, microbeads with different fluorescent labels were used to demonstrate distinct layers.

9. ENGINEERING A NOVEL *IN VITRO* MODEL OF THE BLOOD BRAIN BARRIER

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Introduction. The blood-brain barrier (BBB) is a dynamic interface, regulating the movement of solutes between blood and brain, such as metabolites, molecular waste, and drugs destined for the brain. While the barrier function is predominantly exerted by the endothelial cells (ECs), these properties are not intrinsic to ECs and are induced by the relationships between cell types which exists within the neurovascular unit (NVU) (Liebner et al., 2018). Neither static nor dynamic *in vitro* BBB models fully capture *in vivo*-like conditions, and while coculturing EC monolayers with other NVU cell types has induced better barrier properties, the complexity of these culture conditions detracts from their usefulness for high-throughput drug discovery, testing, and disease modelling. The system we propose utilises material-driven fibrillogenesis by poly(ethyl acrylate) to present an EC monolayer grown on an electrospun PLLA membrane, Which promotes the synergy of growth factor and integrin binding sites available on fibronectin (FN). While there are a handful of papers on electrospun-based BBB *in vitro* models (Bischel et al., 2016; Qi et al., 2018), we hypothesise that an effective *in vitro* model can be created with the use of key growth factors and EC monolayer grown on a PEA and fibronectin-coated electrospun membrane scaffold without the need to establish a co-culture. PLLA electrospun fibres additionally provide a more flexible, biodegradable, and potentially thinner scaffold on which the ECs can grow.

Experimental Methods. Electrospun membrane (both random and aligned fibres) are produced from PLLA 8% solution in hexafluoro-2-propanol and coated with plasma-polymerized PEA (pPEA). Nanofiber morphology and diameter was measured using SEM, TEM and AFM. Inserts to hold membranes in cell culture were designed using AutoDesk Fusion360 and printed on a BCN3d Sigma printer in PLA. Once optimised, immortalised BBB EC cell line, hCMEC/D3, and immortalised human EC cell line, HBEC, were grown on FN-coated membranes in conditions with or without PEA coating, or with additional growth factors added, FGF-1 (100ng/ml). Cell/barrier characterisation includes cell characterisation and tight junction immunofluorescence. iPSC cells provided by the Granata lab at Cambridge University, we additionally shown to express tight junction protein, Claudin-5, a protein proported to be vastly important in the BBB, on the provided pPEA-PLLA membranes.

Results and Discussion. hCMEC/D3 cells were grown on FN-coated glass, un/coated with PEA and with and without FGF-1. Cells grown on PEA/FN conditions showed faster proliferation and lower doubling time. Tight junctions (claudin-5 and occludin staining) were expressed in all conditions, although localisation at cell-cell contacts was increased in the FN/PEA/FGF-1 condition. The cells were then successfully grown on PLLA electrospun membranes under the same conditions, and TEER measurements were taken. hCMEC/D3 cells have low expression of TJs on membranes, as shown by Biemans et al., 2017, and their overall TEER values are considered very low (Eigenmann et al., 2013). This was then tested again using NGF and other growth factors in order to increase barrier characteristics, including exploring arranged electrospun fibres in differing geometries. Ultimately hCMEC/D3 cells were replaced by hiPSC-derived brain endothelial cells and same assays repeated.

Conclusion. We demonstrate that a versatile and tuneable design is capable of inducing barrier characteristics in both hiPSC and immortalised hCMEC/D3 cells. There is room for further optimisation, particularly for the electrospun scaffold and growth factor choice and their combinations, although in the model proves promising even at this early stage. This system offers a promising platform, with prospects for the study of BBB physiology and pathology, as well as a platform for high-throughput BBB drug permeability testing.

References

Biemans et al., J NEUROSCI RES (2017)
Bischel et al., J BIOMED MATER RES A (2016)
Eigenmann et al., FLUIDS BARRIERS CNS (2013)
Liebner et al., ACTA NEUROPATHOL (2018)
Qi et al., ACS APPL MATER INTER (2018)

10.THE ROLE OF PIEZO 1 IN TRANSDUCING VISCOSITY CUES TO THE CELL NUCLEUS

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Introduction. Local dynamic changes in tissue mechanics exert a critical function in pathophysiological processes. For instance, bone formation, degradation and regeneration are modulated by mechanical stresses. At a cellular level, bone tissue cells are directly affected by mechanical changes of their microenvironment. These local cues are converted into electrical or chemical responses via *mechanotranduction*. Piezo 1 (P1) is a mechanosensitive ion channel present in the plasma membrane of various cell types. Its function is to convert mechanical stresses into a biochemical signal which might ultimately target the nucleus **[1].** Recent literature **[2][3]** found that P1 knockout (KO) in osteoblast lineage cells as well as KO mice led to a severe impairment in bone development and growth. Therefore, there is evidence to suggest that the mechanosensitive ion channel P1 is involved in the mechanotransduction process by which bone cells respond to mechanical changes in their environment.

Materials and Methods. In this project, wild type (wt), hP1 overexpressing and hP1 Knock Out (KO) Human Embryonic Kidney (HEK) cells were characterised in terms of P1 expression by means of (Real-time) RT qPCR. Next, they were seeded on flat viscoelastic UV polymerised FN/PEG hydrogels of ranging stiffness (2.5-15 KPa). To understand how the expression of P1 is influenced by the substrate's mechanical cues, such as hydrogel stiffness, the morphology and adhesion of the 2D culture was assessed by immunofluorescence. Furthermore, mechanotransduction was assessed by measuring the mechanoreactive transcriptional factor yes-associated protein (YAP).

Results and Discussion. RT qPCR qualified our three different cell lines by levels of hP1 expression. Next, cell morphology analysis showed cell area and circularity differed between the WT cell line and the P1 KO and overexpressing lines, which suggests a role for this channel in cytoskeleton arrangement. Furthermore, in previous literature **[4]** P1 KO showed to inhibit YAP nuclear translocation, alluding to the role of this transcriptional factor as a downstream signal for P1 channel activity.

Conclusion and outlook. The effect and function of the P1 channel was characterised in relation to elastic substrates of ranging stiffness. This was done in accordance to the previously mentioned literature that related the elasticity of the cells' environment in physiology and pathology; and thus, that of the P1 channel in transducing these elasticity cues to the cell nucleus. We plan to extend the experimental activity by addressing the role of the viscosity of the substrate, other than the elasticity, on the piezo1-mediated mechanotransduction mechanisms. As living tissues behave as viscoelastic solids, where the viscous component is of relevance for cell proliferation and differentiation, this project will aim to investigate and characterise the role of P1 in transducing viscosity cues to the cell in the future exploiting a previously explored platform

[1] *Ridone, P., et al., (2019). Piezo1 mechanosensitive channels: what are they and why are they important. Biophysical Reviews, 11(5), pp.795-805.*

[2] *Sun, W., etal., (2019).The mechanosensitive Piezo1 channel is required for bone formation.eLife, 8.*

[3] *Li, X. et al., (2019). Stimulation of Piezo1 by mechanical signals promotes bone anabolism. eLife, 8.*

[4] *Pathak, M., et al., (2014). Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells. Proceedings of the National Academy of Sciences, 111(45), pp.16148-16153.*

[5] *Bennett, M., etal., (2018). Molecular clutch drives cell response to surface viscosity. Proceedings of the National Academy of Sciences, 115(6), pp.1192-1197.*

11.DEVELOPING THE NANOKICK BIOREACTOR FOR COMMERCIALISATION AND CELL THERAPY

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Nanokicking (NK), the induction of nanoscale mechanical vibrations onto cellular populations, has emerged as a novel methodology of driving osteogenesis of mesenchymal stem cells without the requirement of chemical or biological stimulation (Nikukar et al 2013). Clinically, bone is one of the most transplanted tissues second only to blood (Perez, Kouroupis et al 2018). Approximately 11 million cm^3 of bone grafts is estimated to be used in over 2 million procedures per year (Fernando de Grado et al, 2018) and therefore represents a critical topic in the field of tissue engineering to address the high demand for bone tissue and osteoregenerative treatments. In recent years Nanokicking has developed to fulfil this niche, providing high quality osteoblasts produced within controlled conditions from stem cell populations, currently aiming at producing an ”off the shelf” injectable cell therapy to treat bone related injuries such as non-union fractures.

While current projects, funded by the Sir Bobby Charlton Foundation, look to validate this NK system *in vivo,* preliminarily to the first in man trail in 2021, further foresight must be employed with regards to future large-scale clinical trials. The current iteration of the NK bioreactor, able to produce ~3 million cells per treatment, while feasible for seeding of > $3cm^3$ of bone graft is insufficient for either larger defects, multiple sites of injury or more advanced stages of clinical trial. Therefore, in order to optimise this system for commercial therapeutic treatments the current bioreactor and production methodologies require upscaling in accordance to GMP standards. The aim of this project is to further develop the current nanokicking system in collaboration with industrial partners to establish ideal culture conditions, nanovibrational amplitudes, transportation methods and upscaling production of cells from 2D flat bioreactors to 3D hollow fibre systems able to expand greater numbers of cells, to improve the impact that this technology will have when translated as a commercial clinical cell therapy.

References

1.Nikukar, H., Reid, S., Tsimbouri, P., Riehle, M., Curtis, A. and Dalby, M. (2013). Osteogenesis of Mesenchymal Stem Cells by Nanoscale Mechanotransduction. *ACS Nano*, 7(3), pp.2758-2767.
2.Perez, J., Kouroupis, D., Li, D., Best, T., Kaplan, L. and Correa, D. (2018). Tissue Engineering and Cell-Based Therapies for Fractures and Bone Defects. *Frontiers in Bioengineering and Biotechnology*, 6.
3.Fernandez de Grado, G., Keller, L., Idoux-Gillet, Y., Wagner, Q., Musset, A., Benkirane-Jessel, N., Bornert, F. and Offner, D. (2018). Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management. Journal of Tissue Engineering, 9, p.204173141877681

12. Effects of Peptoid Nanosheets on Stem Cell Culture

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Nanomaterials have attracted great attention in the fields of drug delivery and regenerative medicine. Their unique size not only gives them the properties of both bulk materials and molecular structures but also a high surface to volume ratio. Their size-dependent properties, tunable biochemical characteristics and drug loading capacity are highly desirable in biomedical applications[1].

Unlike nanoparticles and nanotubes, however, less attention has been paid to nanosheets. As a result, it is unclear how these novel nanostructures interact with (stem) cells. In response, we are investigating the interaction of mesenchymal stem cells (MSCs) with "peptoid" nanosheets, formed from peptide-mimetic poly (N-substituted glycine) sequences composed of alternative hydrophobic and ionic side chains,[2] that mimic lipid bilayer membranes (Figure 1). Such nanosheets not only offer the benefits of nanoparticles, but they also display a defined self-assembled structure as ordered as the in vivo protein 2nd structures (Figure 2).

In this work, nanosheet formation is characterized in terms of nanosheets size and number. The stability in various physiologically relevant media is also assessed. The nanosheet-MSC interaction is explored using microscopy imaging (Figure 3), as well as gene and protein expression and mineral studies. Nanosheets appear to be a stiff material, and we hypothesize that they will enhance the MSCs commitment to the osteogenic lineage. It was demonstrated that the nanosheets both increase the metabolic activity of mesenchymal stem cells and have an effect on MSCs differentiation. As a matter of fact, nanosheets increase slightly but significantly the commitment of MSCs to the osteogenic lineage. However, more work is being done to increase and control the effect of nanosheets on MSCs.

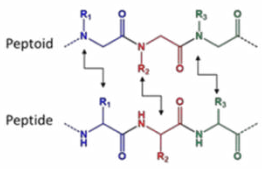

Figure 1, Peptoid vs peptide structure: The side chain is shifted from the α carbon to the nitrogen compared to peptide.

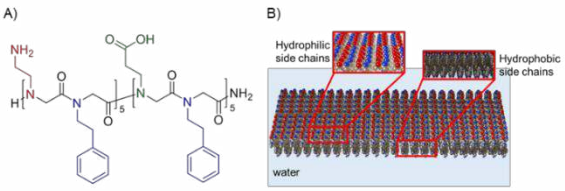

Figure 2, A) Chemical structure of peptoid sequence making up peptoid nanosheets structure. B) Nanosheet crystalline structure: Hydrophobic side chain are buried inside the interior while polar side chain facing the water

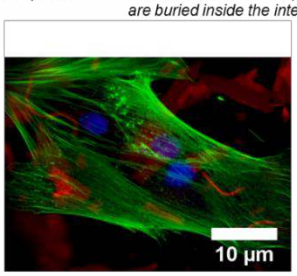

Figure 3, Microscopy picture of MSCs (blue dapi stain the nucleus, green rhodamine stain the actin filaments) with Nanosheets (nile red staining the hydrophobic core).

References:

(1) A. Hasan, M. Morshed, A. Memic, S. Hassan, T.J. Webster, H.E. Marel, *Int. J. Nanomedecine,* 2018, 13, 5637-5655.

(2) K.T. Nam, S.A. Shelb, P.H. Choi, A.B. Marciel, R. Chen, T. Tan, T.K. Chu, R.A. Mesh, B.C. Lee, M.D. Connolly, C.Kisielowski, R.N. Zuckermann, *Nature Materials*, 2010, 9, 454-460.

13.VISCOELASTIC HYDROGELS DRIVE CHONDROGENESIS OF MESENCHYMAL STEM CELLS

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Introduction: While the stiffness of elastic hydrogels has been long recognised to determine human mesenchymal stem cell (hMSC) differentiation, viscous interactions are only recently emerging as powerful regulators of stem cell fate (1). Indeed, viscoelastic hydrogels, where mechanical stresses relax over time, have been shown to promote hMSC spreading and proliferation, as well as formation of an interconnected bone matrix from osteoblasts. On the other hand, elastic hydrogels can inhibit cell proliferation and, in the case of chondrocytes, limit cartilage matrix formation (2). Hydrogels can also be functionalised with peptide motifs to improve hMSC attachment, as well as influence cell fate: for example, chondrogenesis can be controlled by peptide gradients using integrin receptor RGD and cadherin ligand HAVDI (3). However, investigations into how the viscoelastic properties of hydrogels influence the chondrogenic differentiation of hMSCs are lacking. In this work, we investigate whether viscoelastic hydrogels, functionalised with specific peptide combinations, can provide a cellular microenvironment conducive to the chondrogenic differentiation of hMSCs. We have fabricated polyacrylamide (PAAM) hydrogels with variable viscous properties that show consistent elasticity and we have shown differences in hMSC spreading, mechanotransduction and chondrogenesis as a consequence of viscosity.

Experimental methods: Polyacrylamide gels were prepared using different ratios of acrylamide and cross-linker N, N'-Methylenebisacrylamide. Elastic and viscous properties of the hydrogels was measured using AFM and rheology. Human MSCs were cultured on hydrogels functionalised with RGD/HAVDI and analysed by immunoflourescence microscopy for spreading behaviour, as well as markers of mechanotransduction, early chondrogenesis, cartilage matrix, and chondrocyte hypertrophy.

Results and discussions: Nanoindentation and microrheology measurements via AFM showed that the Young's modulus of the polyacrylamide hydrogel family prepared was maintained at ~13 kPa, whilst there was an almost two-fold difference in loss modulus between the least and most viscoelastic gel. This trend was confirmed by rheological measurements, which showed a consistent storage modulus of ~4 kPa (confirming that the gels have equivalent compressive Young's moduli) and a loss modulus ranging from 74 to 164 Pa. Adhering MSCs were studied for their adhesive properties on hydrogels functionalised with RGD/HAVDI, where clear differences in cell spreading and circularity could be observed across the gels as a function of viscosity or peptide ratios. We could observe homogenous distribution of peptides on the gel surface using fluorescently-labelled RGD/HAVDI. Also, differences in mechanotransduction were observed across the viscoelastic gels based on YAP nuclear translocation and lamin A/C:B1 ratio. Early chondrogenesis was investigated, confirming a chondrogenic phenotype on viscous gels with increased SOX9 and decreased RUNX2 expression. Cartilage matrix markers Col2a1 and aggrecan were also upregulated on more viscous gels, while Col1a (fibrocartilage marker) and ColX (hypertrophic marker) were downregulated, suggesting production of neocartilage.

Conclusions: These novel findings show that viscous interactions can be modulated to promote chondrogenesis of MSCs. Gels will be modified to control the interplay between cell-ECM and cell-cell interactions as a strategy to further drive cell fate towards the chondrogenic phenotype. Finally, translation will be tackled by using degradable, PEG-based gels and investigating the effect of their viscoelastic properties on MSCs in 3D cultures.

References:
(1) Chaudhuri O et al. Nat Mater 15:326 (2016).
(2) Lee HP et al. Nat Mater 16:1243 (2017).
(3) Vega S et al. Nat Comm 9 (2018).

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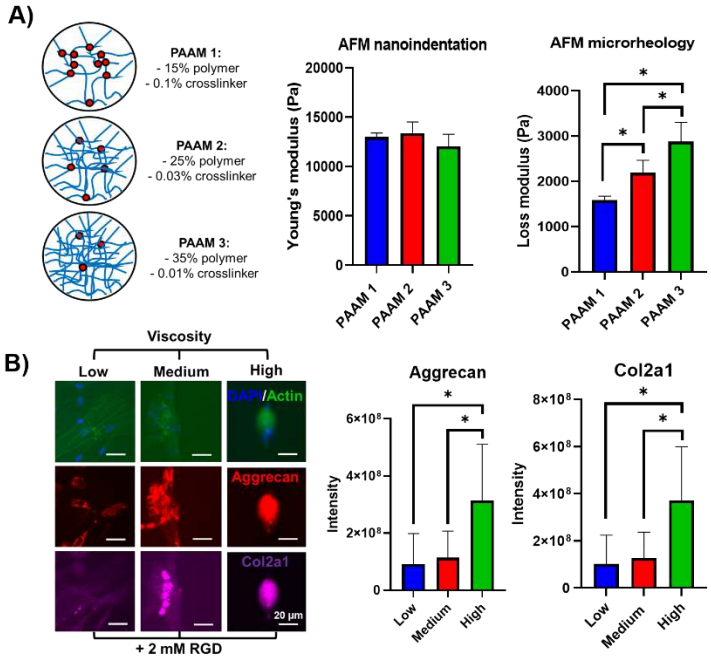
Disclosure of Interest: None Declared
Keywords: Hydrogels for TE applications

Image:



Mechanical properties of PAAM hydrogels and matrix secretion of hMSCs: A) Schematic of PAAM crosslinking and organisation within hydrogel matrix (left), Elasticity measurements by AFM nanoindentation (middle) and viscosity measurements by AFM microrheology (right), n=3, *=p<0.05. **B)** Immunofluorescence of phalloidin, nuclei, aggrecan and Col2a1 for hMSCs attached to surface of viscoelastic PAAM hydrogels functionalised with RGD (left) Quantification of aggrecan (middle) and Col2a1 (right) immunofluorescence using ImageJ and GraphPad Prism software, n=30, *=p<0.05.

14.HYBRID MICROENVIRONMENTS FOR THE *EX-VIVO* EXPANSION OF HAEMATOPOIETIC STEM CELLS

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Introduction. Hematopoietic stem cells (HSCs) have gained traction in research due to their unique capacity to regenerate haematopoiesis in the event of haematological disorders[1]. To achieve their *ex-vivo* expansion, we propose a novel approach based on living biointerfaces that will feature the chemical and structural characteristics of the BM. Our system includes the use of non-pathogenic, genetically-engineered *Lactococcus lactis* biofilms, producing key factors that contribute to HSC phenotype maintenance and proliferation[2]. Additionally, we are mimicking the bone marrow architecture with FN and Laminin functionalised PEG hydrogels and by also incorporating mesenchymal stem cells (MSCs) into the system.
Materials and Methods. Recombinant proteins (CXCL12, TPO, VCAM1, FN)[3] were cloned in the pT2NX plasmid and the constructs were transformed in *L. lactis* using electroporation. For the CD34+ cell experiments, HSCs were seeded on top of *L. lactis* biofilms. After 5 days of incubation at 37°C, 5% CO_2, the HSCs were collected and phenotyped in a flow cytometer. In parallel, the effect of the biofilms on BM-MSCs were tested; MSC spreading and adhesion was evaluated using immunofluorescence and their phenotype was assessed using In Cell Western analysis. To assess the effect of the hydrogels on HSC phenotype maintenance, we engineered 3% and 5% w/v PEG hydrogels, functionalised with 0.3mg/ml of either FN or Laminin. Primary human BM isolated CD34+ cells were seeded at a density of 10^6 cells/ml and were cultured at 37°C, 5% CO_2 for 5 days.
Results and Discussion. We have characterised both protein production in *L. lactis* and the effect of the recombinant cytokines on MSCs and HSCs. Neither cell type seems affected by the presence of bacteria or hydrogel. Furthermore, our results suggest that our system supports HSC phenotype maintenance and can achieve up to 19-fold HSC expansion in 2D experiments. Analysis of the interaction between MSCs and the biofilms has also shown that the MSCs attach and spread on *L. lactis* biofilms and exhibit a niche-like phenotype in response to the recombinant cytokines. This is important as the MSCs could potentially be used as a feeder layer to produce more HSC self-renewal factors. Finally, we report maintenance of the initial HSC population after 5 days in 3% and 5% PEG hydrogels functionalised with Laminin and Fibronectin.
Future directions. Having proof of concept that our system has a promising potential to closely mimic the native BM microenvironment, our next steps will involve a combination of the protein-producing biofilm with the MSC layer and a functionalised hydrogel to create a living material for the *ex-vivo* HSC expansion.

References

1. Schuster, J. A. *et al.* Expansion of hematopoietic stem cells for transplantation: current perspectives. *Exp. Hematol. Oncol.* **1,** 12 (2012).
2. Hay, J. J. *et al.* Bacteria-Based Materials for Stem Cell Engineering. *Adv. Mater.* **30,** (2018).
3. Asada, N., Takeishi, S. & Frenette, P. S. Complexity of bone marrow hematopoietic stem cell niche. *Intl J. of Hematology* **106,** 45–54 (2017).

15.IN VITRO MODELS TO STUDY THE EFFECTS OF NANOVIBRATION AT BONE-SOFT TISSUE INTERFACE

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Mechanical vibrations are being investigated as a potential non-invasive method of promoting bone growth and regeneration in patients with conditions affecting bone density, such as osteoporosis. Vibrations with very high frequency (1000Hz) and nanoscale amplitude (<100nm) have been shown to promote mesenchymal stem cell (MSC) differentiation to osteoblasts, without the need for biochemical inducers associated with high cost and safety concerns. Applied externally, nanovibrations would propagate thought the soft tissues. While the effects of these nanoscale vibrations on MSCs have been repeatedly demonstrated, little is known about how they might affect other types of cells in the tissues surrounding the bone. For this reason, we are making bone models from 3D printed polymer scaffolds and MSCs and soft tissue models from bovine collagen and dermal fibroblasts. We are using qPCR, in-cell western and cell proliferation analyses to examine the effects of nanovibrational stimulation on these 3D cultures. Once they are characterised individually, we will combine them into one bone-soft tissue model, which will allow us to determine the fibroblast response to nanovibrations in an environment mimicking bone-soft tissue interface.

16.Vibrations in space-time to vibrations in bone: the gravitational wave detection technology being used by cell engineers

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The detection of gravitational waves is arguably one of the most significant scientific achievements of the 21st century; as well as confirming a key prediction of Einstein's theory of general relativity, it has provided a wealth of new/enhanced technologies which can benefit the wider scientific community. One such technology, termed nanokicking, involves the precise nanoscale vibration of mesenchymal stem cells at 1 kHz in order to shift them towards an osteogenic lineage. Such a purely mechanical technique, not requiring chemical induction, could be ground breaking for bone graft procedures, treatment of diseases of the bone, and for fracture healing. Repeatability of nanovibrational stimulation across a number of national labs is likely associated with the precision measurement techniques employed in the development of the underlying bioreactor apparatus. This is done through the use of laser interferometry, finite element analysis and piezo technology, all of which have been used in the study and development of gravitational wave detectors. An overview of some of these techniques is presented in relation to the Nanokick bioreactor.

17.Characterisation of transducer arrays for the optimised *in vivo* delivery of nanovibrational stimulation for the treatment of disuse osteoporosis

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Nanovibrational stimulation of mesenchymal stem cells *in vitro* has been demonstrated to promote osteoblastogenesis. To assess the feasibility of this technique for *in vivo applications*, for example as a treatment for osteoporosis, the application of nanoscale vibrations are performed in a preliminary evaluation in a bovine femur. In order to develop a prototype device capable of transmitting the required nanoscale vibrations to the target regions in the bone, two types of transducer set ups, a large single-point transducer and an array of smaller transducers, were used to apply nanoscale vibration into a distal femoral condyle. The vibration amplitudes were measured inside the bone using an accelerometer that was positioned within machined slots within the femur. A scanning laser interferometer was used to precisely map the vibration amplitude on the far surface of the bone. A comparison of the vibration propagation using the two types of transducer device is presented.

18.POLY ETHYLENE (GLYCOL) HYDROGELS AND POLY (ETHYL ACRYLATE) SURFACES USED TO MIMIC THE BONE MARROW NICHE

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INTRODUCTION: Poly ethylene(glycol) (PEG) is a bioinert and biocompatible material that can be used to form hydrogels for 3-dimensional cell culture1,2. Using PEG hydrogels, we can control gel physiochemical properties and composition, such as stiffness and degradability, through various PEG volumes and/or the addition of a degradable crosslinker. Poly ethyl(acrylate) (PEA) can be used as a surface coating which drives fibronectin fibrillogenesis aiding in cell adhesion and allowing synergistic presentation of growth factors3. Here we show a model combining both PEG hydrogels and PEA surfaces used for mesenchymal stem cell (MSC) culture to produce a bone marrow like environment in vitro.
EXPERIMENTAL METHODS: Hydrogels were produced with different PEG volumes (3, 5 and 10 % wt.) and the differences in stiffness shown using rheology. Surfaces were coated in PEA using either plasma polymerisation or UV polymerisation and analysed with x-ray photoelectron spectroscopy (XPS). MSCs were seeded onto PEA coated microbeads which had been treated with FN and in some cases bone morphogenic protein 2 (BMP-2) and neural growth factor (NGF). These beads were then embedded into PEG hydrogels at the point of gel formation completing the model. After several weeks culture in the model cells were analysed to assess their phenotype.
RESULTS AND DISCUSSION: Rheology shows that by altering the volume of PEG in the hydrogels we can tune the stiffness of the gels to that desired for the model. Stiffness has been shown to have an influence on cell behaviour including driving cell differentiation down a specific lineage. XPS analysis shows the presence of PEA on the surface of both plasma coated samples and those that underwent UV treatment. Results of MSC culture in this model indicate the ability of the cells to maintain a naive phenotype, as well as committing osteogenic lineage when exposed to the growth factor treated surfaces. This allows us to create a co-culture from a single cell type that represents multiple cell types found within the bone marrow niche.
CONCLUSION: By combining these two materials, PEG hydrogels & PEA surfaces, we are able to control physiochemical properties of the model. This allows us to better control the cells within the model progressing toward a more accurate in vitro representation of the bone marrow niche. Further work with the introduction of haematopoietic stem cells could help us understand the interactions between these cell type in vivo.
ACKNOWLEDGEMENTS: We would like to thank EPSRC for grant EP/P001114/1 funding S. Donnelly's PhD. We also thank Carol-Anne Smith for technical assistance.
REFERECES: [1] Zhu J. Biomaterials. 2010; 31(17): 4639-4656. [2] Raeber G et al. Biophys. J.2005; 89(2): 1374-88 [3] Llopis-Hernandez V et al. Sci. Adv.2016; 2(8)

19.CAN MSC-DERIVED EXTRACELLULAR VESICLES INDUCE BREAST CANCER CELL DORMANCY IN THE BONE MARROW?

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Breast cancer cells (BCC) favourably metastasise to the bone marrow niche, where they interact with resident mesenchymal stem cells (MSCs) and potentially become dormant. How this interaction facilitates cancer cell dormancy is poorly understood, however recent evidence has postulated the role of MSC-derived EVs in the onset this dormancy. Here, we study the effect of MSC-derived extracellular vesicles (EVs) on a BCC line, MCF7. We compare MSC-derived EVs under both control conditions and MSCs challenged by wounding (i.e. injury-activated MSCs), to determine whether MSC-derived EVs can stimulate MCF7 cell proliferation or dormancy.

MCF7s were grown in standard 2D monolayer and 3D spheroid culture (generated via ultra-low attachment surface microplates), to verify whether our 2D findings translated to a more appropriate 3D microenvironment. Cells were incubated with both control and scratched MSC-EVs. Thereafter, MCF7 cell cycle was analysed by performing a Fluidigm qPCR assay for a range of cell cycle and global cell signalling genes. Cell viability and metabolism (MTT assay) were also assessed.

From this study, we noted a general decrease in expression of MCF7 cell cycle, as well as cell signalling genes involved in a variety of pathways, supporting the hypothesis that MSC-EVs may influence BCC dormancy. Next, we will take a metabolomics approach that will assess the content of MSC-derived EVs, in order to determine whether any specific metabolites can be used to control MCF7 proliferation and/or dormancy.

20. 3D MICROENVIRONMENTS TO ENGINEER BIOLOGICAL-INSPIRED VASCULARIZED BONE MODELS

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Introduction. Bone is a highly vascularized and dynamic microenvironment that undergoes a constant remodelling process. Thus, engineering *in vitro* bone models require the provision of the signals experienced during this remodelling. Among them, bioactive signal molecules and physical cues can be provided to ultimately regulate cell fate. In the present work, the development of a 3D bone model is introduced as a system that presents the vascular endothelial growth factor (VEGF) to the cells tethered to poly-ethyl acrylate (PEA) to induce vascularization. This polymer has been shown to allow solid-phase presentation of GFs through fibronectin (FN) surface-binding mimicking the stem cell microenvironment and allowing low and controlled dose administration of signals1. In addition, nanovibrational stimulation or 'nanokicking' (NK) has been applied to the system in order to study the effect of mechanical forces in mesenchymal stem cells (MSCs) within the current model. This mechanical stimulation has shown a strong osteogenic response in two-dimensional (2D) and three-dimensional (3D) conditions 2. This work is focused also in the use of the aforementioned PEA/FN surfaces to present neuropeptides to cells creating a real physiological bone micro-environment in which neuro, osteogenic and vasculogenic cues are present. Thus, with the use of a single material, different responses can be achieved at the same time for bone tissue engineering.
Methods. Substrates were made with PEA by plasma polymerization (pPEA) and characterized using X- ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and interferometry. To evaluate osteogenic and angiogenic potential of the construct, monocultures and co-cultures of MSCs and endothelial cells (ECs) were carried out onto different surfaces after 7 and 21 days. VEGF and neuropeptide SP have been presented as matrix-bounded to FN in order evaluate any synergistic effect during the osteogenic-vasculogenic coupling. Fibrin gels were added to these systems to study vascular formation *in vitro.* To trigger osteogenic response, samples were nanostimulated during the cell culture period time by a bioreactor at 1000 Hz and 30nm. Immunostaining for CD31 was used to evaluate protein expression and tubular formation. VE-cadherin have been used to study the effect of nanovibrations on the formation and stability of vessel-like structures. At gene level, PCR studies of osteogenic and angiogenic markers were carried out after 21 days. As a 3D model, pPEA/FN-beads have been investigated within the gels as an ultra-low growth factor/neuropeptide support for vascularized bone models.
Results. Nanokicking (NK) seems to enhance CD31 expression in 2D (when VEGF is matrix bound) and in the presence of fibrin gels (when VEGF is in a soluble form). However, an effect on vascular formation is not really noticeable. In long-term co-cultures of MSCs/HUVECs with GFs and fibrin gels, vascular-like networks were only noticed in presence of VEGF, matrix-bound or soluble. However, a minimal migration was detected after 21 days by either MSCs or HUVECs. Greater VE-cadherin expression was shown in 2D for VEGF conditions proposing that nanovibrations are involved in endothelial cells mechanotransduction. More specifically, VE-cadherin complexes may act as conformation specific force transducers in a mechanically integrated, force-sensitive network and its expression is increased in 2D under nanovibrations. To improve MSCs-HUVECs interaction, the afore mentioned system was rebuilt as a 3D model using PEA/FN beads. This approach seems to be a more reliable system, although futher work is needed on its implementation with the current model. On the other hand, SP seems to bind to FN but is still needed the use of other techniques to understand this interaction. Regarding angiogenic response, SP seems to be involved in early endotelial cell arrangements.

Conclusions. The material-based system represents a model for angiogenesis and osteogenesis. With an *in vitro* angiogenesis assay, CD31 analysis suggests that nanovibrations themselves do not seem to induce tubule formation but does it appear to increase CD31 expression. On the other hand, VE-cadherin expression has been shown to be nanovibrations-dependent. Also, the incorporation of molecules, such us GFs or neuropeptides, could provide a biological-inspired model that mimics the coupling of angiogenesis and osteogenesis. The versatility of the system and its optimisation regarding the co-culture experiments will produce a direct interaction between both type of cells, a critical step in long-term vessel maintenance and stability.

References

1. Llopis-Hernández, V. et al. Sci. Adv.2,e1600188 (2016).

2. Tsimbouri, P. M. et al. Nat. Biomed. Eng. (2017).

21.Collagen type I functionalization using recombinant fragments of fibronectin: A novel approach to bind and present physiological amounts of BMP2.

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Introduction: One of the major challenges in tissue engineering is to deliver growth factors in an efficient for prolonged time periods [1]. Collagen type I, a material often used in tissue engineering, does not have a high affinity and binding capacity to growth factors. Researchers have been incorporating growth factors such as Bone Morphogenic Protein 2 (BMP2) into collagen sponges for bone regeneration for several years [2]. Lower affinity of collagen required high doses of BMP2, which are related with a higher risk of cancer [3]. The aim of this study is to incorporate lower doses of BMP-2 into collagen-based materials that can be presented locally. We have genetically engineered bacteria which express a new recombinant fragment that combines the collagen binding region (FNI6-9II1-2) and the growth factor binding region (FNIII12-14) of fibronectin [4]. The fragment can be isolated and incorporated stably into a collagen-based material (e.g. collagen hydrogel).
Experimental Method: The custom fragment (FN6-9II1-2-link-III12-14) was expressed in BL21Star E. Coli bacteria utilizing a pet100 vector. Bacteria growth conditions were optimised, and the expressed protein was purified using a Nickel column. Fragment attachment to collagen was verified by immunostaining collagen hydrogels after submerging them in FN6-9II1-2-link-III12-14 containing solution as well as hisTag ELISAs. Untreated collagen gels and gels treated with shorter, FNIII12-14 fragments, were used as a comparison. Growth factor binding and release was verified by submerging the treated collagen gels in solutions of 2, 1, 0.5, 0.1 μg/ml Alexaflor488 labelled BMP-2 and consequently in PBS. Aliquots of the supernatant were removed and analysed at set points up to 7d. Human Mesenchymal Stem Cells (hMSCs) were cultured and seeded on the material groups to check if the cells are viable on the system for the upcoming osteogenic differentiation experiments.
Results: The results suggest that FN6-9II1-2-link-III12-14 successfully binds collagen and is retained up to 95% while FNIII12-14 fails to do so. The former is also better at binding and retaining small amounts of BMP-2 more effectively than the latter and the control group for all concentrations used. HMSC viability and attachment experiments on peptide treated collagen materials were successful, indicating that the first osteogenic differentiation studies (von Kossa, OPN, OCN) can now be carried out.
Conclusion: Collagen functionalised with FN6-9II1-2-link-III12-14 is an innovative method of absorbing and retaining physiological amounts of BMP2 to promote osteogenic differentiation of hMSCs. This system is not only limited to BMP2 but can work with other growth factors e.g. PDGF.

[1] S. Werner and R. Grose, “Regulation of Wound Healing by Growth Factors and Cytokines,” *Physiol. Rev.*, vol. 83, no. 3, pp. 835–870, 2003.
[2] M. Geiger, R. H. Li, and W. Friess, “Collagen sponges for bone regeneration with rhBMP-2,” *Adv. Drug Deliv. Rev.*, vol. 55, no. 12, pp. 1613–1629, 2003.
[3] H. S. Yang, W. La, and B. Kim, “Comparison between heparin-conjugated fibrin and collagen sponge as bone morphogenetic protein-2 carriers for bone regeneration,” *Exp. Mol. Med.*, vol. 44, no. 5, pp. 350–355, 2012.
[4] M. Martino and J. A. Hubbell, “The 12th – 14th type III repeats of fibronectin function as a highly promiscuous growth factor-binding,” pp. 4711–4721, 2019.

22.THE ASSESSMENT OF BREAST CANCER CELL INVASION INTO THE BONE MARROW

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Abstract

Breast cancer is the most common form type of cancer in women, it is able to disseminate and metastasise to different areas within the body, notably the bone marrow. Within the bone marrow and the Mesenchymal Stem Cell (MSC) niche the breast cancer cells (BCCs) enter a state of dormancy and can reside for many years evading traditional cancer treatments. The dormant state of these cancerous cells can be reversed, and the cells reactivated, thus causing re-emergence of the cancer usually as a secondary bone marrow tumour. It is now known due to recent research that EVs secreted by both the cancerous cells and the resident MSCs play an active role inducing dormancy of the invading BCCs. Contained within exosomes are enzymes, proteins, DNA, mRNA and miRNA; these cargos allow EVs to mediate various signalling pathways such as the paracrine and autocrine pathways. It has been shown that in cancers EVs play a crucial role as drivers of metastasis and tumour progression by supressing immune response, remodelling the extracellular matrix, activating fibroblasts and inducing drug resistance. MCF7 (breast cancer cell line) cells will be treated with MSC-derived EVs in order to investigate the metastatic potential of EVs.

A PROGRAMMABLE AND NON-INVASIVE BIOMATERIAL TO AID WOUND HEALING USING PDGF-BB IN AN EFFICIENT MANNER

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Abstract

Introduction: We present a major step towards an effective, safe therapeutic solution in the management of complex wounds. Wound healing is promoted by a number of chemokines and growth factors (GFs) including platelet-derived GF-BB (PDGF-BB). Current GF products used for wound healing have a large associated cost and potential adverse effects (e.g. PDGF containing Regranex® warns not to use more than 3 times due to complications such as death secondary to malignancy. To safely unlock the potential of GFs and chemokines, appropriate interactions of these molecules with the extracellular matrix are critical to regulation of signalling and effectiveness. Our approach utilises a simple polymer, poly(ethyl acrylate) (PEA) that promotes the self-organization of fibronectin (FN) into biological nanonetworks. After a simple adsorption onto PEA, the FN exposes FN-FN binding sites allowing spontaneous network formation and this unravels the FN molecule to reveal cell adhesive RGD domains, GF binding domains (FNIII12-14) and chemokine binding domains (FNI1-5). We have previously shows effectiveness of this system to deliver BMP2 for bone regeneration1.

Results and discussions: Fibroblasts were able to express more fibronectin and intregrin α_5 when in contact with the PDGF-BB presented through the FN on PEA. They were also able to close the wound faster in 2D (Figure1,b) and, when cultured in a transwell, fibroblasts growing with a PDGF-coated sample in the bottom well were able to migrate more due to chemoatractant effects. When the functionalised surfaces were tested with keratinocytes, an increase in the signalling of the PDGF-BB pathway was observed when in contact with the PDGF-BB bound to FN coated samples, indicating that the GF was efficiently presented to the cells due to the FN network. When the individual keratinocytes velocity was analysed, it showed that the cells were migrating more when the sample had PDGF adsorbed onto the FN network on PEA. When the system was studied in 3D, fibroblasts and keratinocytes in the epidermal model were closing the wound quicker and keratinocytes were expressing more maturation markers.

Conclusions: We have develop a system that can be functionalised to promote fibroblast migration and keratinocytes differentiation enhancing wound healing using very small doses of GF. PDGF-BB was adsorbed on the material and allowed the cells to close the wound *in vitro* faster promoting wound healing signalling, as shown in 2D and 3D models. This material could be a good and safer candidate for diabetic ulcers, reducing the currently used amounts of PDGF-BB in clinics.

References/Acknowledgements:

1. Science Advances 26 Aug 2016: Vol. 2, no. 8, e1600188

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AETIOLOGY AND RISK FACTORS OF DEEP TISSUE INJURY EXPLAINED

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Background: The soft tissues covering bony prominences often encounter unique biomechanical challenges. Although not intended to tolerate high loads and deformation, they become part of the weight-bearing structure in surgical and bedfast patients, wheelchair users and lower-limb amputees. Consequently, deep soft tissue layers may be damaged, resulting in Deep Tissue Injury (DTI). However, the underlying aetiological and risk factors are subject to ongoing debates.

Material and Methods: A review of the state-of-the-art knowledge on DTI is presented. This is based on a scoping review across the databases Pubmed, Ovid Excerpta Medica, and Scopus, which analysed 16 English-language studies on (1) the population-specific aetiology of transtibial amputees, (2) risk factors, and (3) methodologies to investigate both.

Results: The results indicate that DTI development is dependent on a complex interplay of mechanical, anatomical, and physiological factors acting on different organisational levels, from the external environment over soft tissue through to single cells. Whilst the loading conditions play a major role, they are strongly influenced by individual determinants. However, methodological limitations, high inter-patient variability, and small sample sizes complicate the interpretation of outcome measures. Additionally, fundamental research on cell and tissue reactions to dynamic loading and on its influence on the vascular and lymphatic systems is missing.

Conclusion: We therefore recommend increased interdisciplinary research endeavours with a focus on closing the identified gaps to widen our understanding of DTI. The results have the potential to initiate much-needed advances in surgical, clinical, and prosthetic practice and inform future pressure ulcer classifications and guidelines.

HYBRID LAMININ-BASED HYDROGELS FOR EFFICIENT PRESENTATION OF GROWTH FACTORS

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INTRODUCTION: Hydrogel systems can be engineered with the aim of regenerating different tissues (e.g. musculoskeletal, bone, vascular and cardiovascular). Protein-based hydrogels are appealing for their structural designability, specific biological functionality, and stimuli-responsiveness. Here, we present 3D Poly (ethylene glycol)-laminin (PEG-LM) hydrogels for delivery of growth factors in a controlled manner [1][2][3]. By combining LM with PEG, hybrid biomaterials, containing both natural and artificial components, are generated, allowing precise control of mechanical properties.

EXPERIMENTAL METHOD: 3D laminin-based hydrogels containing different concentrations of PEGylated human laminin with acrylates were crosslinked via photopolymerisation with two- or four-arm acrylate and a protease-degradable peptide (VPM). Human mesenchymal stem cells from bone marrow (hMSCs) and human umbilical vein endothelial cells (HUVECs) with different (bone and vascular related) growth factors were incorporated into the laminin hybrid hydrogels to evaluate cell cytotoxicity, study the controlled release of the growth factors and their phenotypical potential to driven different cell linages (Figure 1). hMSCs differentiation was assessed by immunofluorescence and qPCR by analyzing the expression of osteopontin (OPN) and osteocalcin (OCN), two osteogenic differentiation markers, at different time points, whereas the vascularization assessment was made by immunofluorescence.

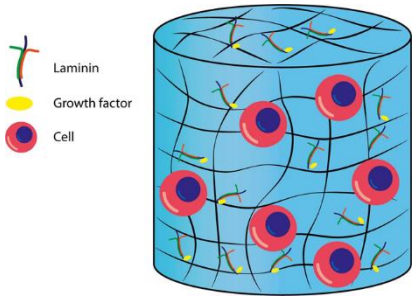
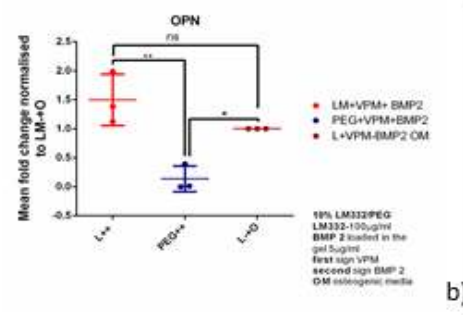


Fig. 1 Schematic representation of cells and growth factors encapsulated in 3D Hybrid Laminin hydrogels.

RESULTS AND DISCUSSIONS: We studied the ability of the hybrid PEG-laminin hydrogels to promote regeneration of different tissues (e.g. bone, vascular and nervous) by delivering growth factors in a controlled manner. To do this, 3D laminin-based hydrogels with tuneable stiffness and different degradability ratios were successfully synthesized with the aim to mimic the native extracellular matrix (ECM). Other properties such as growth factor release kinetics and biocompatibility of the hydrogels were evaluated to determine if our system could be a suitable biomaterial for other types of tissues. Immunofluorescence and qRT-PCR results of hMSC encapsulated in 3D LM/PEG hydrogels loaded with 5ug/ml BMP-2 showed an upregulation of osteopontin expression compared to the positive control at 14 days (Figure 2a). Also, LM/PEG hydrogels loaded with 8ug/ml vascular endothelial growth factor (VEGF) showed a greater potential to promote endothelial cell sprouting after 4 days in culture.

a)



b)

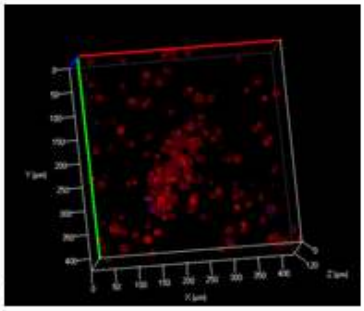


Fig. 2 a) Gene expression analyses for a marker of osteogenesis (OPN) in hMSC encapsulated in LM/PEG hydrogels for 14 days (with 5 ug/ml BMP2) and b) representative 3D reconstructions of HUVECs within LM/PEG hydrogel at days 4 (with 8ug/ml VEGF).

CONCLUSIONS: These novel hydrogels combine proteins with synthetic materials and can be used to engineer in vitro tissue models. We report on the development and characterization of hybrid 3D laminin hydrogels with tuneable mechanical and degradable properties and highly efficient growth factor presentation for potential tissue regeneration applications such as bone and vascular tissues.

REFERENCES: [1] M. Salmerón-Sánchez and M. J. Dalby, "Synergistic growth factor microenvironments," *Chem. Commun*., vol. 52, no. 91, pp. 13327–13336, 2016. [2] A. Aubrey T. Franciscoa, Priscilla Y. Hwanga, Claire G. Jeonga, Liufang Jinga, Jun Chenb and B. Lori A. Settona, "Photocrosslinkable Laminin-Functionalized Polyethylene Glycol Hydrogel for Intervertebral Disc Regeneration," *Acta Biomater*, vol. 6, no. 9, pp. 2166–2171, 2008. [3] J. Ishihara, A. Ishihara, K. Fukunaga, P. S. Briquez, J. A. Hubbell, Nature Comm.; doi: https://doi.org/10.1101/25948.

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ENGINEERED HYDROGELS USING FULL-LENGTH FIBRONECTIN THAT SEQUESTER AND PRESENT GROWTH FACTORS

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Growth factors (GFs) have potential clinical value, especially in regenerative medicine. Their rapid clearance in vivo makes necessary the development of material-based strategies that control their sequestration and release, to lower their effective dosage.

Bioinspired materials aim to exploit natural extracellular matrix (ECM) features as they sequester GFs and support regenerative processes. Thus, the ECM can act as a GF reservoir that coordinates the availability of GFs through ECM-GF interactions. However, natural ECM-derived matrices (e.g. Matrigel) that are widely used to culture cells in vitro and can recapitulate some ECM functions, suffer from lot-to-lot variability and lack of controlled physical properties. Therefore, there is a need to develop rationally planned synthetic matrices that also mimic ECM roles.

Fibronectin (FN), an ECM protein, promiscuously binds GFs, including the bone morphogenetic protein 2 (BMP2, osteogenic) and the vascular endothelial GF (VEGF, angiogenic). FN also presents its GF-binding site next to a cell adhesive site and, the simultaneous binding of both by the cell activates synergistic signalling pathways, which boosts the effect of GFs alone [1, 2].

Here we report the development of synthetic, 3D hydrogels that incorporate full-length FN to provide an alternative to natural ECM-derived matrices. The use of full length FN enables ultra-low dose and solid-sate presentation of GFs for tissue regeneration purposes.

FN was covalently linked to a synthetic poly(ethylene) glycol (PEG) polymer using Michael-type addition reaction via maleimide-thiol chemistry. FN-based Hydrogels (FNPEG) were synthesised with different amounts of PEG and with different ratios of degradable:non-degradable thiolated crosslinkers. FNPEG gels were loaded with either VEGF or BMP2 for in vitro and in vivo studies. FNPEG gels were loaded with endothelial cells and VEGF to study angiogenesis in vitro and in vivo using the chick chorioallantoic membrane (CAM) assay. FNPEG gels loaded with BMP2 were tested in vivo using a murine critical-size (non-healing) defect model.

FNPEG hydrogels were synthesised with controlled stiffness and degradability by varying the amount of PEG and degradable crosslinker added, respectively. The system was cytocompatible after cell encapsulation. VEGF-loaded FNPEG hydrogels released less GF compared to PEG controls without protein, being able to retain up to 50% of the VEGF loaded. Moreover, VEGF uptake assays showed the ability of FNPEG hydrogels to bind more GF than the controls with no FN.

We also studied the angiogenic effect of FN-VEGF interaction using 3D in vitro models, loading endothelial cells-coated microcarriers and finding enhanced sprouting compared to non-FN gels. This system has also shown its angiogenic performance in a CAM assay.

Finally, bone growth in vivo was tested using a murine non-healing radial defect, demonstrating that FNPEG hydrogels loaded with low amounts of BMP2 can promote bone regeneration.

Overall we present a new synthetic system that incorporates full-length FN. The system is fully tuneable (e.g. stiffness, degradation) and can bind GFs. The exploitation of FN-GF interactions shows that this system can be used for tissue engineering applications as it promotes angiogenesis in vitro and in vivo using VEGF and also, supports bone growth in vivo with low doses of BMP2.

References:

1. Llopis-Hernández V, et al., Science Advances, 2016.
2. Martino M, et al. Science Translational Medicine, 2011.

ACRYLATE PLASMA COATED 3D PRINTED POLYCAPROLACTONE SCAFFOLDS FOR BONE REGENERATION

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Three dimensional (3D) porous scaffolds with favourable osteogenic ability and mechanical properties are promising candidates for bone repair and regeneration. Scaffolds geometry, porosity and topography in combination with chemical composition and surface coating are known to influence cell attachment, proliferation, differentiation and subsequently osteointegration of biomaterials. Taken this evidence base into account, the aim of our study is to evaluate the bioactivity of a novel 3D printed polycaprolactone (PCL) scaffold with nanometer thick polymer coating designed for use as a bone implants to heal the mind blast injury. Here we used an inductively coupled plasma system to modify 3D scaffold with thin coatings of poly (ethyl acrylate) (PEA). Our lab has previously shown the outstanding functional properties of PEA, that induce a fibrillar conformation of fibronectin (FN) adsorbed on its surface, biomimetically exposing its integrin and growth factor-binding domains, and in turn allowing an efficient and synergistic presentation of growth factors in vitro and in vivo [1, 2].
The efficiency of plasma polymerisation as well as FN adsorption and interaction with bone morphogenetic protein-2 (BMP-2) on PEA coated PCL scaffolds were evaluated. We have then studied the in vitro ability of PEA coated PCL scaffolds to facilitate cell proliferation and osteoblast differentiation when the scaffolds were subjected to varying scaffolds pore geometry accomplished by manipulating the advancing angle between printed layers. Alkaline phosphatase assays, PCR analysis, immunofluorescence and calcium mineralisation studies revealed that the PCL scaffolds coated with PEA, FN and BMP-2 enhanced the osteogenic differentiation of hMSCs. Subsequently, modified PCL scaffolds were implanted subcutaneously into rat model to evaluate ectopic bone formation and early stage vasculature after 12 weeks. We propose that our functionalised 3D scaffolds exhibit potential technique for bone regenerative therapies.

References

1. V. Llopis-Hernández, etal. Material-driven fibronectin assembly for high-efficiency presentation of growth factors. Sci Adv. 2016 Aug 26;2(8)
2. Z.A. Cheng, etal.,. Nanoscale coatings for ultralow dose BMP-2-driven regeneration of critical-sized bone defects. Advanced Science 2018, 6.