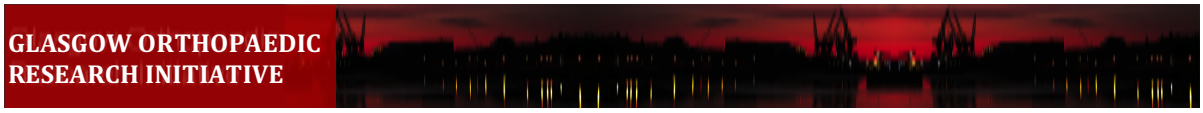

GLORI 2018

Meeting of the Glasgow Orthopaedic Research Initiative on 23rd of February at the Teaching and learning centre in QEUH.

Programme:

09.15 Coffee

Session 1 – chairs Dr Monica P Tsimbouri and Mr David Shields

09.30 Welcome from **Prof R M Dominic Meek,** *Consultant, in Orthopaedics and Trauma Surgery, QEUH, Glasgow.*

09.35 **Dr Mathis Riehle** and the story of CCM

09:40 Invited talk - **Prof Matt Dalby,** *Professor of Cell Engineering, GU*
Title: "Nanokick: stimulation of osteogenesis by mesenchymal stem cells using a nanovibrational bioreactor"

09:55 Andrew Fagan, Licor

10.00 Invited talk - **Prof Manuel Salmeron-Sanchez,** *Chair of Biomedical Engineering, GU*
Title: "Engineered bmp-2 based systems for efficient regeneration of bone critical-size defects"

10:20 Ben Arnold, ThermoFisher

10.25 Invited talk - **Dr Mathis Riehle,** *Reader CCE, GU*
*Title: "*Cells, drugs and bioengineered constructs to aid peripheral nerve repair"

10.45 Invited talk - **Dr Helen Wheadon,** *Non-Clinical Senior Lecturer Cancer Biologist, GU*
Title: "The role of bone marrow morphogenic signals in sustaining chronic myeloid leukaemia"

11.05 Coffee

Session 2 – Chairs Mr Rob Silverwood and Dr Cristina Gonzales

11.35 Invited talk **Prof Sue Barnett,** *Professor of Cellular Neuroscience*, **GU**
Title: "Study of Proliferate™, a Novel Polymer for central nervous system (CNS) Repair"

12.00 Keynote Speaker**- Prof Cosimo De Bari** *Professor of Translational Medicine & Honorary Consultant Rheumatologist, Aberdeen*
Title: "The regenerative biology of the synovial joint"

12.45 Invited talk -**Dr Rob Wallace,** *RA, Edinburgh*
Title: "Microarchitecture and The Time-Dependent Mechanical Response of Bone"

13.10 Invited talk **Dr Sanjay Gupta,** *Consultant Orthopaedic, GRI*
*Title: "*Multicentre collaborative research – the journey so far"

13:30 Lunch and posters

Session 3 – chairs Dr Virginia Llopiz-Hernandez and Dr Tom Hodgkinson

14.30 Invited talk- **Mr Neal Millar,** *Academic Consultant Orthopaedic Surgeon, GU*
Title: "Inflammatory mechanisms in tendon disease: *a translational journey"*

14.55 **Mr Rob Silverwood ,** *Clinical Fellow, GU*
Title:" The development of a 3d osteoprogenitor culture model for investigating future osteoporosis therapies"

15.05 Invited talk **Mr Jon Clarke,** *Orthopaedic Consultant and Orthopaedic Research Lead at GJNH*
*Title: "*Advances in the management of knee disorders"

15:25 **Dr Ewan Ross,** *RA, GU*
*Title: "*Nanotopographies induce changes to mesenchymal stem cell metabolism and promote multipotency – subtle changes have profound effects"

15:35 **Dr Oana Dobre,** *RA, GU*
*Title: "*Hybrid Laminin hydrogels for bone regeneration"

15:45 Invited talk – Prof **Stuart Reid** *(Univ. of Strathclyde)*
Title: "Exploiting collaborative ventures between astrophysics and biomedical research: nanokicking stem cells, engineering of anti-biofouling surfaces, and medical capnography".

16:05 Posters and prizes

16:30 Meeting ends

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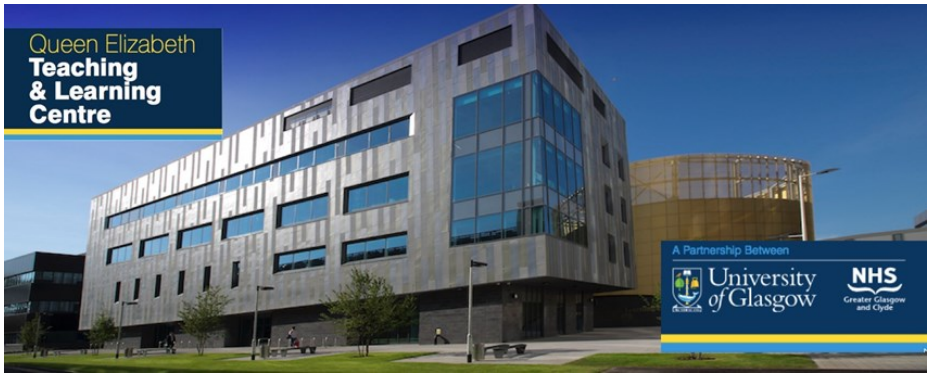
Travel Information

This link has a map and travel advice on how to get to the campus

http://www.nhsggc.org.uk/patients-and-visitors/main-hospital-sites/queen-elizabeth-university-hospital-campus/teaching-and-learning-centre/travel-information/



Previous Next

Public Transport : Public transport provides direct access to the Queen Elizabeth University Hospitals and allows interchange with the subway, other bus services and the train at Govan, Partick or in the City Centre. Arrival Square is located in the centre of the Queen Elizabeth University Hospitals site and can be accessed from Govan Road and Hardgate Road.

Busses run from Partick station every 10 min from stance1 or 2 bus numbers 8, 17 or the airport bus 77. Journey time 10-15min and fare cost £2.30 single trip.

Local Rail or subway stations: Cardonald train station and Govan Subway station which is 1.3 miles from the Hospital campus

Cycling: Cycling is a great way to travel to the hospitals. There are shared access routes to the Hospitals campus and cycle hire facilities at Govan Cross, Paisley Road Toll and Partick Interchange. Designated visitor cycle parking and a public bike hire station are located at Arrivals Square. Visit www.cyclestreets.net to get the route that suits you.

Walking : The hospitals can be reached on foot from Cardonald train station and Govan Subway station which is 1.3 miles from the Hospital campus. The Hospitals are also accessible by dedicated tunnels for pedestrian and cyclist access to the Clyde Tunnel. Please use the online journey planner to plan your route www.walkit.com

Car Parking: There are drop off and pick up bays at Arrival Square for both cars and taxis. At times the car parks and surrounding roads can be busy and you should consider other travel options such as the bus if it is a suitable alternative. A number of disabled parking bays are available on campus. Car parking is free and

attendants are available to provide advice if required. Please note there is a 4 hour maximum stay. Visitors to the Teaching & Learning Centre should note that park and ride facilities are available throughout Glasgow www.spt.co.uk/park-ride

Rail : The hospitals are located around 1.2 miles from Cardonald Train Station with regular buses serving the Hospitals. Cardonald Train Station is managed by Scotrail and is on the Inverclyde line linking Wemyss Bay, Gourock and Glasgow Central. To plan your journey online please use www.travelinescotland.com journey planner or visit www.scotrail.co.uk

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QEUH Site Map

Oral Presentations

NANOKICK: STIMULATION OF OSTEOGENESIS BY MESENCHYMAL STEM CELLS USING A NANOVIBRATIONAL BIOREACTOR

Matthew J Dalby

Centre for Cell Engineering, University of Glasgow

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Bone grafts are one of the most commonly transplanted tissues. However, autologous grafts are in short supply, and can be associated with pain and donor-site morbidity. The creation of tissue-engineered bone grafts could help to fulfil clinical demand and provide a crucial resource for drug screening. Here, vibrations of nanoscale amplitude provided by a newly developed bioreactor will be discussed that can differentiate a potential autologous cell source, mesenchymal stem cells (MSCs), into mineralized tissue in 3D. Further, nanoscale mechanotransduction can stimulate osteogenesis independently of other environmental factors, such as matrix rigidity. This is demonstrated by generating mineralized matrix from MSCs seeded in collagen gels with stiffness an order of magnitude below the stiffness of gels needed to induce bone formation in vitro. Our approach is scalable and can be compatible with 3D scaffolds.
The bioreactor, the Nanokick, provides nanoscale vibrations of 30 nm amplitude at 1000Hz to MSC cultures. In 2D, osteogenesis occurs through a cell-adhesion/ROCK dependent mechanism. However, in 3D, ion channels, specifically TRPV1, a transient receptor potential cation channel, stimulates RUNX2 driven osteogenesis through a PKC / β-catenin pathway. Looking towards developing the technique to produce bone graft, we are using collagen foams as well as gels to provide a stiffer cell environment that can be easily handled and can also incorporate e.g. bone mineral granules.

We acknowledge support from BBSRC, EPSRC, MRC and Find A Better Way.

Nikukar, H. et al. Osteogenesis of mesenchymal stem cells by nanoscale mechanotransduction. ACS Nano 7, 2758-2767 (2013).

Tsimbouri, P.M. et al. Stimulation of 3D osteogenesis by mesenchymal stem cells using a nanovibrational bioreactor. Nature Biomedical Engineering 1, 758-770 (2017).

ENGINEERED BMP-2 BASED SYSTEMS FOR EFFICIENT REGENERATION OF BONE CRITICAL-SIZE DEFECTS

Manuel Salmeron-Sanchez

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There is a pre-clinical bottleneck of potential advanced regenerative therapies due to over-engineering, use of novel chemistry unlikely to gain regulatory approval, and/or complex presentations of biologicals. Thus, while there are many exciting new biomaterials emerging in the literature, translation is slow. This means that current regenerative approaches are reliant on e.g. high doses of growth factors (GFs), such as BMP-2 in bone regeneration, which can have serious side effects. Here we describe translation of an ultra-low dose GF technology with high bioactivity based on a simple polymer, poly(ethyl acrylate) or PEA, that triggers spontaneous organization of fibronectin and GFs, allowing synergistic mesenchymal stem cell adhesion and BMP-2 receptor activation. To translate this technology we, for the first time, employ plasma polymerization of the polymer onto 2D and 3D substrates to give *in vitro* demonstration of cell signal enhancement and then to drive full healing of a critical-size bone defect as demonstrated in vivo. Further, we demonstrate safety and efficacy in a veterinary patient, a large Münsterländer with a non-union fracture of the humerus that had repeatedly failed to heal and which was due to be amputated. In this case, we coated bone allograft with plasma-polymerized PEA and effected full bone regeneration in 6 weeks. This is a first full translation of an advanced ultra-low dose GF treatment.

CELLS, DRUGS AND BIOENGINEERED CONSTRUCTS TO AID PERIPHERAL NERVE REPAIR

Mathis Riehle

Centre for Cell Engineering, University of Glasgow

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Peripheral nerve repair has yet to catch up with the progress other in areas of surgery and medical device development. The best possible type of repair of larger gaps continues to be by using an autologous nerve transplant, which requires a second surgery and leads to donor site morbidity. To further the use of cells and devices we developed a combinatorial approach where adipose derived stem cells are combined with a micro fabricated porous scaffold to repair a critical size gap in a preclinical rat model. In addition, we develop strategies to pattern cells in two and three dimensions off and on the device and use acoustic or electroactive devices to support the regenerative phenotype in neurons and glia. Ideally these devices and cell transplants would be supported by small drugs that further enhance regeneration, or maintain the regenerative phenotype of glia for longer. Signalling pathways that are involved in this response are growth factors, mTor and AMPK; where we found interesting material structure dependent interactions between stimulation or inhibition of growth factor pathways, mTor and AMPK if neurons had been grown on structured substrates.
We hope to translate our findings into better devices and improved combinatorial strategies for peripheral nerve repair.

THE ROLE OF BONE MARROW MORPHOGENIC SIGNALS IN SUSTAINING CHRONIC MYELOID LEUKAEMIA

[1]Helen Wheadon, [1]Gillian Horne, [3]Parto Toofan, [2]Caroline Busch, [2]Catherine Berry, [4]Victoria Campbell, [5]Heather Morrison, [1]Mhairi Copland.

[1]Institute Cancer Sciences, Paul O'Gorman Leukaemia Research Centre, University of Glasgow, [2]Institute Molecular, Cells and Systems Biology, University of Glasgow, [3]MRC Centre for Regenerative Medicine, University of Edinburgh, [4]NHS Lothian, [5]NHS Greater Glasgow and Clyde.

Email: Helen.Wheadon@Glasgow.ac.uk

Chronic myeloid leukaemia (CML) results from a genetic change in a haemopoietic stem cell (HSC), leading to a hierarchical clonal stem cell disease, with the expanding leukaemic stem cell (LSC) population sustaining the malignancy within the bone marrow (BM) niche. The cells express the constitutively active tyrosine kinase BCR-ABL, which causes rapid cell division and leukaemia. Therapy involves tyrosine kinase inhibitor (TKI), which effectively inhibits BCR-ABL, thereby controlling CML. However, TKI doesn't eliminate the LSC population, therefore patients are not cured and require life-long therapy. This phenomenon of disease persistence under therapy, suggests BCR-ABL-independent mechanisms are being exploited to sustain the survival of LSC. Increasing evidence suggests that the BM microenvironment plays a pivotal role in the initiation and progression of the leukaemia. Of particular interest are the morphogens, growth factors implicated in embryogenesis, developmental haemopoiesis and homeostasis. Microarray analysis, comparing chronic phase (CP), accelerated phase (AP) and blast crisis phase (BP) CML LSCs and progenitor populations to normal HSCs and progenitors, indicated that the Notch, Wnt, TGF superfamily and Hedgehog (Hh) pathway are highly deregulated in CML. To investigate this further, we profiled mesenchymal stem cells (MSCs) from normal donors and CML, CP (n=12), myeloid BP (n=11), and lymphoid BP (n=5) stem and progenitor populations, for gene components of Wnt, Notch, Hedgehog, and BMP pathways. Data indicates that self-renewal pathways were highly deregulated between CP and BP with statistically significant upregulation in Wnt components in myeloid BP compared to CP. Targeting the pathways using small molecule inhibitors indicate that the BMP, Hh and Notch pathways are viable therapeutic targets in combination with TKI and that Wnt upregulation is preventing Notch activation in myeloid BP-CML. These findings highlight the complexity of self-renewal pathway interaction especially in progressive disease.

THE REGENERATIVE BIOLOGY OF THE SYNOVIAL JOINT

Cosimo De Bari *Professor of Translational Medicine & Honorary Consultant Rheumatologist, Aberdeen*

MICROARCHITECTURE AND THE TIME-DEPENDENT MECHANICAL RESPONSE OF BONE

Wallace RJ[1], Xie S[2], Manda K[2], Simpson H[1], Pankaj P[2]

[1] University of Edinburgh Department of Orthopaedics and Trauma
[2] University of Edinburgh Institute of Bioengineering

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The strength of a structure is determined by both the arrangement of, and the mechanical properties of the material it is made from. Aging and bone disease can affect trabecular microarchitecture, altering the arrangement, size and density of trabeculae. Changes to the distribution of material will influence the response to mechanical load.

Bone reacts to load in a non-linear and time dependant fashion; these properties can describe the response to impact, slow vertebral collapse and implant loosening and are therefore highly relevant to enable improved orthopaedic designs.

Using high resolution micro-CT imaging and our creep/relaxation mechanical testing protocol we evaluated the trabecular microarchitecture and determined the influence these properties have on the time-dependant properties of bone.

We observed that there is a strong and significant relationship between key time-dependant mechanical properties and trabecular microarchitecture. We also found that irrecoverable strain (residual deformation) exists at all load levels and only correlates with microarchitecture at levels of strain associated with yield. This implies that viscoelastic processes occur at multiple hierarchical levels. However, the larger irrecoverable strains that are associated with phenomena such as screw loosening occur on a scale that is influenced by microarchitecture.

Our research is now focused on investigating the effect that osteoporosis treatment and obesity have on microarchitecture. We aim to utilise this data, incorporating the time-dependent relationships, to provide an input to finite element models, thereby enabling the design and evaluation of implants that will provide better long-term fixation into these compromised bone types.

MULTICENTRE COLLABORATIVE RESEARCH – THE JOURNEY SO FAR

Sanjay Gupta**,** *Consultant Orthopaedic, GRI*

INFLAMMATORY MECHANISMS IN TENDON DISEASE: ***A TRANSLATIONAL JOURNEY***

Neal L Millar PhD FRCSEd (Tr&Ortho)

Senior Clinical Lecturer in Orthopaedics, Honorary Consultant Orthopaedic Surgeon, University of Glasgow

Email: Neal.millar@glasgow.ac.uk

Neal Millar is an Academic Consultant Orthopaedic Surgeon specialising in shoulder surgery and tendon injuries having completed fellowships in Sydney and New York. His research interest lies in investigating the molecular pathophysiology of tendinopathy; an overuse injury, characterised by tendon pain and weakness with a significant burden of disease. His laboratory's research focuses on the immunopathogenesis and translational immunobiology of soft tissue musculoskeletal diseases including tendinopathy. Additionally, he runs a specialist 'One stop' complex tendon clinic in the NHS focused on improving the treatment of tendinopathy. His work has been generously funded by the Wellcome Trust, Arthritis Research UK, The Scottish Funding Council, Chief Scientific Office Scotland and Scottish Enterprise grants. He is Chief Medical Officer and Co-Founder of the University of Glasgow spin out venture, Causeway Therapeutics which is developing a novel microRNA injectable therapy for tendon disease.

This talk will highlight the role of inflammation in tendinopathy and how mechanistic dissection of the immunobiology of this disease has led to two novel translational tendon therapies which will enter human clinical trials.

THE DEVELOPMENT OF A 3D OSTEOPROGENITOR CULTURE MODEL FOR INVESTIGATING FUTURE OSTEOPOROSIS THERAPIES

R.K. Silverwood[1,2], M. Mullin[3], R.M.D. Meek[1,2], M.J. Dalby[1], C.C. Berry[1]

[1]University of Glasgow, Centre for Cell Engineering, Glasgow, United Kingdom, [2]Queen Elizabeth University Hospital, Trauma & Orthopaedics, Glasgow, United Kingdom, [3]University of Glasgow, Electron Microscopy Facility School of Life Sciences MVLS, Glasgow, United Kingdom

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Osteoporosis represents an increasing burden to healthcare systems worldwide, and their associated fractures are expected to double in incidence by 2050. Current therapies have proven inadequate, and new, targeted therapies are required. A consistent and reliable three-dimensional (3D) model is required to perform high throughput drug screening, in order to successfully develop improved therapies. Multicellular spheroids have long been established in research fields such as cancer, yet there remains a paucity of information on 3D spheroid cultures for osteoprogenitor cells, which are vital to musculoskeletal research. This study will analyse 3 spheroid-forming techniques with human osteoprogenitor cells to identify the optimal culture conditions.

Human bone marrow aspirates, taken at time of total hip replacement, were utilised. The adherent cells were cultured, which gave rise to the osteoprogenitor population. Three spheroid techniques were assessed; hanging drop (HD), ultra-low attachment (ULA) and magnetic levitation techniques. Spheroids were generated using several cell seeding densities and cultured up to 21 days; analysis included cell viability, morphology (light microscopy) and scanning/transmission electron microscopy at time-points 1,7 and 21 days.

The HD and ULA techniques consistently produced circular spheroids, with viable cells, which increased in diameter with increasing cell number. However, the ULA method formed stable spheroids faster and more efficiently. The magnetic levitation technique formed spheroids of a maximum diameter, regardless of cell density, with clear internalisation of the nanoparticles visible on electron microscopy, but suffered areas of cell death within the spheroid. Electron microscopy demonstrated cell-cell interactions within all models.

The ULA technique rapidly produces stable and uniform osteoprogenitor spheroids which remain viable in long term culture.

The ULA model can be adopted as a standard 3D model to allow comparison of both osteoprogenitor cells from healthy and osteoporotic patients. This will contribute to the development of targeted therapies.

STUDY OF PROLIFERATE™, A NOVEL POLYMER FOR CENTRAL NERVOUS SYSTEM (CNS) REPAIR.

Susan Barnett[1], Sara Hosseinzadeh[1], Julia M Edgar[1], Don Wellings[2], Mathis Reihle[3], John S. Riddell[4].

[1]Institute of Infection, Immunity and Inflammation, University of Glasgow, UK; [2]SpheriTech, Runcorn, Cheshire, UK; [3]Centre for Cell Engineering, Institute of Molecular, Cell and Systems Biology, University of Glasgow, UK; [4]Institute of Neuroscience and Psychology, University of Glasgow, UK

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The poor repair that follows CNS injury leads to permanent disabilities for which effective treatments are limited. Several approaches are used to promote CNS repair including cell transplantation and advance rehabilitation, however the lesions become walled off by an astrocytic scar preventing repair. Overcoming the limitations to regeneration/plasticity in the CNS remains the greatest challenge to spinal cord repair following injury. Two key barriers are: (i) the physical gap left by the injury, across which axons must extend and ultimately reconnect, and (ii) the glial scar – the inhibitory milieu perpetuated by reactive glia filling and surrounding the site of trauma. One promising therapeutic strategy for the repair of spinal cord is the implantation of biodegradable scaffolds into the injury site to stabilise the injury and act as a bridge for regrowth, regeneration, plasticity, and reconnection. In collaboration with Spheritech Ltd, we have tested a microporous biodegradable polymer scaffold as a candidate for CNS repair termed Proliferate™. Data will be shown on the ability of proliferate to support growth and differentiation of neural cells both in vitro and in vivo.

ADVANCES IN THE MANAGEMENT OF KNEE DISORDERS

Jon Clarke, *Orthopaedic Consultant and Orthopaedic Research Lead at GJNH*

NANOTOPOGRAPHIES INDUCE CHANGES TO MESENCHYMAL STEM CELL METABOLISM AND PROMOTE MULTIPOTENCY – SUBTLE CHANGES HAVE PROFOUND EFFECTS.

Ross EA[1], Turner LA[1], Saeed A[2], Reynolds PM[2], Burgess KEV[3], Meek RD[1,4], Gadegaard N[2], Oreffo ROC[5], Dalby MJ[1].

[1]*Centre for Cell Engineering, Institute of Molecular, Cell and Systems Biology, CMVLS, University of Glasgow, Glasgow, UK*
[2]*Division of Biomedical Engineering, School of Engineering, University of Glasgow, Glasgow, UK*
[3]*Scottish Polyomics Facility, Wolfson Wohl Cancer Research Centre, CMVLS, University of Glasgow, Garscube Estate, Glasgow, UK.*
[4]*Department of Trauma and Orthopaedics, Queen Elizabeth University Hospital, Glasgow, UK*
[5]*Centre for Human Development, Stem Cells and Regeneration, Faculty of Medicine, University of Southampton, Southampton, UK.*

Email: Ewan.Ross@glasgow.ac.uk

Naïve, multipotent mesenchymal stem cells (MSC) are a key cell product for patient therapy. Their ability to regulate immune responses, haematopoiesis as well as their potential for tissue regeneration mean they have become a key therapeutic tool for cell-based therapies. However, the quality of the naïve MSC is crucial to its function. Over time in culture MSCs differentiate into mature stromal cells, losing their immunosuppressive and haematopoietic stem cell (HSC) supporting abilities. The Centre for Cell Engineering has previously demonstrated that growing MSC on surfaces consisting of a square pattern of nanopits (SQ) results in maintaining their naïve phenotype, with increased expression of multipotency markers and delayed differentiation into mature stromal cells. Data presented here reveals that binding to the SQ surface results in subtle changes to MSC metabolism, with an increase in glycolysis as revealed by heavy labelled glucose tracing. Modifying MSC metabolism with inhibitors recapitulates the SQ surface driven phenotype, maintaining their immunosuppressive activity and upregulating multipotency markers. Importantly, this change in metabolism has a direct functional impact on MSCs. Using the SQ nanotopography to promote the naive MSC phenotype, a novel BM niche model has been developed to provide the necessary signals to promote HSC expansion in the laboratory. We therefore demonstrate that nanotopographical surfaces can be used to investigate biochemical and metabolic changes required to promote the HSC supporting phenotype of MSCs. This system will allow examination of the soluble factors and cell-cell contacts produced by the naïve MSC to promote HSC expansion.

3D HYBRID LAMININ BASED HYDROGELS FOR BONE REGENERATION

Oana Dobre[1], Sara Trujillo-Muñoz[1], Virginia Llopis Hernández[2], Matthew Dalby[2], Manuel Salmeron-Sanchez[1]

[1]Division of Biomedical Engineering, University of Glasgow, UK [2]Centre for Cell Engineering, Institute of Molecular Cell and Systems Biology,University of Glasgow, UK

Email: *oana.dobre@glasgow.ac.uk;*

Hydrogel systems can be engineered with the aim of regenerating different tissues (e.g. musculoskeletal, bone 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]. By combining LM with PEG, hybrid biomaterials, containing both natural and artificial components, are generated, allowing precise control of mechanical properties.
3D Laminin based hydrogels consist of different concentrations of human laminin isoforms, PEGylated to introduce acrylate into the system. The hydrogel is cross-linked via photopolymerisation with two or four arm acrylate and a protease-degradable peptide (VPM). Human mesenchymal stem cells from bone marrow (hMSCs) and different (bone related) growth factors were incorporated into the Laminin hybrid hydrogels to evaluate cell cytotoxicity and study the controlled release of the growth factors (see Figure 1).

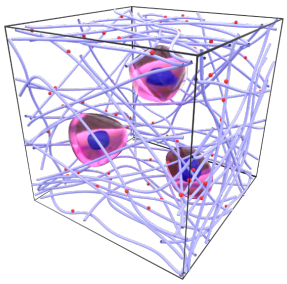

Figure 1. Schematic representation of cells and growth factors encapsulated in 3D Hybrid Laminin hydrogels.

We were investigating the possibility of the hybrid laminin hydrogels to promote regeneration of different tissues (e.g. bone) by delivering growth factors in a controlled manner. In order to do this, 3D Laminin based hydrogels with tuneable stiffness and different rate of degradability were successfully prepared to mimic the native extracellular matrix (ECM).
Other properties such as growth factor release, and biocompatibility of the hydrogels were evaluated to determine if our system could be an alternative biomaterial for bone regeneration or other tissue regeneration.
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.
[1]M. Salmerón-Sánchez and M. J. Dalby, *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, *Acta Biomater*, vol. 6, no. 9, pp. 2166–2171, 2008.

This work was completed with support from the Engineering and Physical Sciences Research Council (EPSRC) Grant EP/P001114/1.

EXPLOITING COLLABORATIVE VENTURES BETWEEN ASTROPHYSICS AND BIOMEDICAL RESEARCH: NANOKICKING STEM CELLS, ENGINEERING OF ANTI-BIOFOULING SURFACES, AND MEDICAL CAPNOGRAPHY.

Stuart Reid
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The detection of gravitational waves in 2015, which was heralded as the scientific breakthrough of the century, required the advancement of numerous technologies to enable laser interferometric measurement to be almost entirely limited by quantum physics effects. This talk will describe how technology and skills developed within the gravitational wave astronomy community have contributed to the advancement of biomedical and cell engineering. Firstly, the use of precision measurement and modelling technologies have enabled the use of nanovibrational stimulation to promote osteogenesis from mesenchymal stem cells (MSCs), for the first time providing a cheap, scalable method to meet clinical demand for bone graft. Secondly, diamond-like carbon coatings, which were doped with germanium, have been shown to provide an environmentally friendly, non-toxic, cheap, and chemically resistant/mechanically robust coating with significant anti-biofouling properties. Finally, optical interference coatings have been developed and applied to CO2 gas sensors manufactured by local SME, Gas Sensing Solutions Ltd, and evaluated for use in capnography monitoring during procedural sedation and analgesia.

We acknowledge support from the EC, STFC, BBSRC, EPSRC, the Society for Chemical Industry, the Royal Society, and Find A Better Way.

Robertson, S. N. et al., Investigation of the antimicrobial properties of modified multilayer diamond-like carbon coatings on 316 stainless steel. Surface and Coatings Technology, 314, 72-78 (2017).

Fleming, L. et al., "One-dimensional photonic crystals for eliminating cross-talk in mid-IR photonics-based respiratory gas sensing," Proc. SPIE 10103, Terahertz, RF, Millimeter, and Submillimeter-Wave Technology and Applications X, 1010318 (2017).

Nikukar, H. et al. Osteogenesis of mesenchymal stem cells by nanoscale mechanotransduction. ACS Nano 7, 2758-2767 (2013).

Tsimbouri, P.M. et al. Stimulation of 3D osteogenesis by mesenchymal stem cells using a nanovibrational bioreactor. Nature Biomedical Engineering 1, 758-770 (2017).

NANOSCALE MANIPULATION OF BONE CELLS: HEALTHY AND CANCEROUS

L.Lee[1], G. Dutour-Provenzano[1], P. Young[1], D. Meek[2], A. Mahendra[3], S.Reid[4], P. Childs[4] M.J. Dalby[1] and M.P. Tsimbouri[1]

[1]Centre for Cell Engineering, University of Glasgow, [2]QEUH, Glasgow, [3]GRI, Glasgow, [4]Strathclyde University, Glasgow

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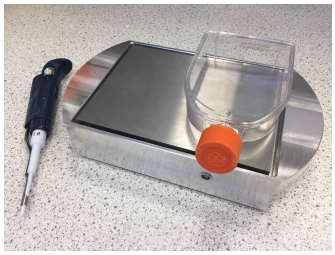
Mechanical stimulation has been explored as an avenue for cancer therapy. Recently, research has focused on cell interactions with nano features of the extra cellular environment, and the consequent mechanotransduction of information from external stimuli through integrin receptors, into intra-cellular biochemical signalling events. We have developed a new technology Nanokicking that could be used to slow down the progression of osteosarcomas (OS). The nanokicking (NK) bioreactor is a platform containing piezo actuators that generate and send nano-vibrations at a frequency of 1 kHz with an amplitude of 30 nm ± 5 nm, into the cells, to influence changes in their growth and metabolism. NK was primarily used to differentiate mesenchymal stem cells (MSC) into osteoblasts by mimicking the physical vibration of the bone in-vivo. With only nano-stimulation, MSCs express bone related genes and display an osteoblast phenotype in 2D and 3D [Tsimbouri et al, 2017].

Our hypothesis is that Nanokicking stimulation will slow down the progression of primary human OS tumours and at the same time support normal cell growth and differentiation. In order to examine the effect of the nanokicking technique on osteosarcoma, primary OS tumour cells and BM stromal cells from healthy donors, were stimulated by a nanokicking bioreactor that stimulated them at a frequency of 1 kHz and 30 nm amplitude for a period of 3 and 7 days. The gene and protein expression was assessed using biomarker expression from a specific apoptosis inhibition pathway: surviving, ezrin, stat3. MSC differentiation was also examined. The gene expression was determined by qRT-PCR and the protein expression by In-Cell Western and immunostaining. Cell metabolic activity was also examined and indicated a reduction in key metabolic pathways in the NK OS samples. Nanokicking at this frequency supported normal stromal cells growth and differentiation while OS cells showed reduced pro-survival gene expression, growth and metabolism. Hence, 'nanokicking' should be further investigated as it may have potential to offer a chemical-free method for differentially regulating healthy cell growth and cancer cell death.



BONE CANCER RESEARCH TRUST

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Tsimbouri MP et al. Nature biomedical engineering 1, 758, 2017.

PERICYTE METABOLIC MECHANISMS TO REGULATE A BONE MARROW NICHE-LIKE PHENOTYPE *IN VITRO*

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Pericytes are a key cell type of the bone marrow niche. They are multipotent and line the endothelium differentiating into mesenchymal stem cells (MSCs) and CXCL12 abundant reticular cells. They act in a support role for haematopoietic stem cells (HSCs) and have immune modulatory and inflammatory functions. When in a support role, they secrete growth factors and chemokines that maintain self-renewal and support HSCs. In culture, however, as with MSCs, they lose their niche phenotype. Here, using a poly ethyl-acrylate (PEA) system on which fibronectin (FN) forms ordered networks, thus allowing growth factor tethering[1], we aim to investigate the metabolic phenotype and mechanisms required for pericytes to maintain niche phenotype.

We show that addition of a collagen gel (noting that soft gels can support expression of niche markers such as nestin[2]) supports a niche-like MSC phenotype when used with a bone-marrow like PEA environment. Immunocytochemistry was then used to show a degree of hypoxic response in this system, with similar levels of activated HIF1α observed compared to no gel in 1% oxygen tension. Correspondingly, we observed an increase in levels of glycolytic enzyme lactate dehydrogenase (LDH), indicative of a switch to an anaerobic metabolic profile. Metabolite-wide analysis revealed agreement in down-regulation of metabolites involved in oxidative phosphorylation. The key genes and signalling pathways involved in this hypoxia response to soft collagen gels was investigated through genome-wide transcriptomic analysis and integration of this with metabolite data.

The anaerobic phenotype corresponds to a maintenance of pericyte niche/support phenotype. This can have large implication for production of pericytes *in vitro* that can be used to support e.g. tissue engineered construct implantation via enhanced anti-inflammatory and immune modulatory properties.

Acknowledgements:
This work was supported by grant BB/N018419/1 (BBSRC) and an EPSRC studentship. We thank Carol-Anne Smith for technical support.

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FIBRONECTIN-BASED HYDROGEL SYSTEMS AS NEW 3-DIMENSIONAL MICROENVIRONMENTS FOR TISSUE REGENERATION.

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Hydrogel systems are of growing interest as ECM mimics, due to their intrinsic and controllable properties (e.g. water content, stiffness); furthermore, these systems can be tailored with biologically active ligands (e.g. cell-adhesion or protease-degradable peptides). In order to sophisticate hydrogels as ECM mimics, significant efforts have been made to incorporate proteins or protein fragments, providing binding domains for different molecules, which peptide ligands lack. To this end, our work focuses on the formulation of hydrogels based on one of the major constituents of the ECM: fibronectin (FN). FN is a glycoprotein that presents binding sites for heparin, collagen, other FN molecules and growth factors (GFs). It has been shown that the exploitation of GF-FN synergisms can alter cell behaviour (e.g. improve cell migration, proliferation or differentiation).
In this work, we have developed two strategies to covalently link FN to synthetic (polyethylene glycol, PEG) and natural (hyaluronic acid, HA) polymers to form 3-dimensional hydrogel systems. FN-PEG hydrogels were formed using a Michael-type addition reaction that takes place at physiological pH and temperature. Using this approach, FN was incorporated up to 1 mg/mL. FN-HA hydrogels were fabricated via a photoinitiated thiol-ene reaction using a norbornene-modified HA. In this case, FN was tethered up to 2 mg/mL. Both systems showed cytocompatibility of murine C2C12 cells and human mesenchymal stem cells at 7 days, respectively. These two systems based on FN could be used to further exploit cell-FN-GF interactions in 3-dimensional cultures while being able to control other physicochemical parameters such as the mechanical properties of the environment.

CELL-IMPRINTED SUBSTRATES AS A CONTROL FOR STEMNESS, DIFFERENTIATION AND COMPOSITION OF STEM CELLS

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The use of stem cells in modern medicine is becoming more frequent and exciting with each passing year. The controlled differentiation and retained stemness of these cells is of great interest in the current climate and the use of mechanotransduction as a cheaper, more efficient alternative to chemical signalling is a growing field of study. This project intends to test the efficacy of PDMS negatively imprinted with mesenchymal stem cells (MSCs) as a substrate for mechanically retaining stemness of MSCs *in vitro*. Experiments will also be performed using negative imprints of other cell types such as osteoblasts to measure the effect of simple mechanical forces on the cells in directing differentiation. The accuracy and viability of the imprint topographies will be analysed using SEM, AFM and contact angle measurements, while the progress and effectiveness of this approach to control differentiation, self-renewal and cellular constitution will be analysed at gene and protein levels through immunostaining, in-cell westerns and qRT-PCR. This project follows on from previous work detailing the potential for cell-imprinted substrates as a reliable and effective approach for regenerative therapies.

ENGINEERING OSTEOGENIC COATINGS ON PEEK - EFFICIENT BMP2 MICROENVIRONMENTS

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Material-based strategies seek to engineer synthetic microenvironments that mimic the characteristics of physiological extracellular matrices for applications in regenerative therapies, including bone repair and regeneration. In our group, we have identified a specific chemistry, poly(ethyl acrylate) (PEA), able to induce the organization of fibronectin (FN) into fibrillar networks upon simple adsorption of the protein from a solution. This process has been called material-driven fibrillogenesis due to its similarity to the physiological one, leading to enhanced cellular response, in terms of cell adhesion and differentiation. Recently, we have exploited these FN networks to capture and present growth factors (GF) in combination with the integrin binding domain of FN to promote bone healing, after finding that fibrillar conformation of FN adsorbed on PEA favours the simultaneous availability of the GF binding domain (FNIII12-14) next to the integrin binding region (FNIII9-10). The combined exposure of domains improved the osteogenic differentiation of mesenchymal stem cells (MSCs). A higher expression of bone markers was found when BMP2 presented from the material interface versus its soluble administration in the culture media *in vitro*. The potential of this system as a recruiter of GFs was also investigated in a critical-size bone segmental defect in mouse. The synergistic integrin-GF signalling, induced by fibrillar FN, promoted bone formation *in vivo* with lower BMP2 doses than current technologies.[1] Furthermore, we optimized the system for its potential use in translational research, seeking to enhance the bioactivity of materials already used in clinics applications (e.g. poly ethyl ether ketone, PEEK). PEEK samples were dry sprayed using PEA, which improved the differentiation of MSCs towards osteogenic phenotypes. We have been able to apply a functional coating on PEEK and proved that this coating has an osteogenic effect on hMSC, as improves their extracellular matrix adsorption, cell adhesion and cell differentiation. We ended up developing polymer and protein coatings having the potential to improve osteointegration after implantation and being able to amend the PEEK surface properties by themselves. The material-driven FN fibrillogenesis provides a new translational strategy to efficiently reduce the GF doses administrated in bone regenerative therapies.

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INVESTIGATING LEUKAEMIC STEM CELL: OPPORTUNE REMODELLING OF THE BONE MARROW MICRO-ENVIRONMENT

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Chronic myeloid leukaemia (CML) is characterised by the proliferation of leukaemic stem cells (LSCs) in the bone marrow (BM). The subsequent deregulation of normal haematopoietic stem cell (HSC) activity, propagated by LSCs, is a key stage in myeloid leukaemia. Recent evidence indicates that LSCs achieve this via modification of the BM microenvironment (niche) to their advantage, whilst impairing normal haematopoiesis. To date it has proved difficult to target LSCs with current therapies and to study exactly how LSCs manage to dominate and alter the niche. In the healthy BM mesenchymal stem cells (MSCs) and HSCs reside together in the niche, where they interact closely and maintain their stem cell properties via self-renewal. We aim to adapt our established artificial 3D model of the BM niche, with the additional co-culture of HSCs and LSCs. The model includes the formation of MSC spheroids embedded in medical-grade collagen to mimic properties comparable with human BM. This will enable the study of cell-cell interactions within a niche-like environment and the processes the LSCs undertake in order to remodel the BM to their advantage. We then aim to use our model to assess potential small molecules and drug treatments in combination.

COLLAGEN CALCIUM PHOSPHATE COMPOSITE DEVICES FOR BONE AUGMENTATION

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Bone injuries are quite common among people and arise from multiple sources such as disease or sporting incidents. However, if a bone defect in humans exceeds 10mm in diameter, then the body is unable to heal on its own without assistance. Assistance is often provided in the form of tissue engineered scaffolds. In recent years advances in bone tissue engineering and scaffold design have inspired a switch from traditional materials such as metal and ceramics to new innovative composite biomaterials. These attempt to mimic the natural structure of bone which on a sub-nanostructural level is made up of collagen and hydroxyapatite like mineral. The aim of the project is to optimize the production of a novel, ion-doped, calcium phosphate reinforced, collagen bone scaffold, characterize its material properties, carry out in vitro cell culture studies and ready it for initial in vivo testing.

COMPOSITE BONE TISSUE ENGINEERING SCAFFOLDS PRODUCED BY COAXIAL ELECTROSPINNING

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Recently, modifications have been made in the basic electrospinning process to improve the quality and the functionality of the resulting fibres. Coaxial electrospinning has gained attention for tissue engineering applications, where two different polymers or composites are delivered independently through a coaxial emitter and drawn to produce core-sheath fibrous structures that are capable of encapsulating bioactive agents and drugs within the core layer. This study aims to produce bioactive scaffolds for bone tissue engineering made of core/ shell fibres via coaxial electrospinning. The core of the fibres is composed of biodegradable PCL polymer while the shell layer is composed of a mixture of PLA and hydroxyapatite. PLA and PCL are used due to their degradability, biocompatibility and high mechanical strength while hydroxyapatite particles are incorporated to buffer the local pH decrease produced by polymers degradation and to improve cell adhesion and osteoconductivity. Optimisation of the scaffolds included testing the morphology of the resulted fibres, mechanical properties, in vitro dissolution and bioactivity, and cell culture.

ENGINEERED BIOFILMS BASED ON NON-PATHOGENIC BACTERIA FOR EX VIVO HEMATOPOIETIC STEM CELL (HSC) EXPANSION

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Hematopoietic stem cells (HSC) reside in the bone marrow and can restore a fully functional hematopoietic system when transplanted. They can differentiate into cells of all mature blood lineages. HSC transplantation has been used in the clinic to treat pathologies such as leukemia and other cancers, and in bone marrow failures or after radiotheraphy. In most cases, the source for HSCs is autologous or with HLA compatible donors. The issue with HSC transplantation is the amount of CD34+ cells required, in the order of 3-4 million cells per kilogram. HSC are rare, 0.01% in the bone marrow, so it is desirable to expand them ex vivo to achieve a successful transplantation. Out of their bone marrow niche, HSC quickly differentiate to committed progenitors and then to the lymphoid of myeloid lineage, so it is important to replicate a bone marrow-like microenvironment to achieve this ex vivo expansion.

This work focuses in the use of bone marrow-specific biochemical factors such as cytokines like angiopoietin 1, CXCL12, VCAM-1, SCF and thrombopoietin. All of them need to be produced in a controlled fashion to achieve this objective. The use of recombinant gram-positive non-pathogenic bacteria has been documented for MSC differentiation, since they do not interfere with the differentiation process. These engineered bacteria produce, in situ and under tight control, the proteins required for stem cell fate control.

Our aim is to develop engineered biofilms that produce these cytokines and proteins required to mimic the bone marrow microenvironment, in a physical setting matching the bone marrow mechanical properties with the same biochemical cues displayed to the HSC. The advantages of this system are their simplicity over MSC-HSC co-cultures and the in-situ, controlled production of cytokines versus the use of externally supplemented cytokine cocktails, which allows for a tighter protein expression control.

INVESTIGATION OF MECHANOTRANSDUCTIVE PATHWAYS IN HUMAN FIBROBLASTS

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Cells convert mechanical stimuli into specific biochemical signals through a process called mechanotransduction, which requires an intact cytoskeleton. Mechanotransduction features heavily in the adaptation of the skin to various naturally occurring forces such as various strains and compression. Skin fibroblasts respond to mechanical forces by differentiating into myofibroblasts, which express α-smooth muscle actin (αSMA), a contractile intracellular protein. αSMA helps the skin adapt to force application and it is expressed after the nuclear localisation of myocardin-related transcription factor A (MRTF-A), which takes place as a result of mechanically-induced RhoA activation. In this investigation, an in-house built stretching device will be used to apply slow unidirectional strain to human dermal fibroblasts seeded on a polycaprolactone (PCL) membrane (12% w/v). Previous results from this lab demonstrated a 13% increase in the nuclear/cytoplasmic ratio of MRTF-A after application of stretch for 24h and failed to show any αSMA expression. In this experiment, strain of the same rate and magnitude will be applied for 12, 24 and 48h to investigate the strain duration dependency of MRTF-A nuclear localisation and αSMA expression, demonstrated through fluorescent microscopy and automated image analysis. Additionally, blebbistatin will be used to inhibit myosin II, thus disturbing acto-myosin motility and cortical tension, while the LPA receptor agonist Oleoyl-L-α-lysophosphatidic acid sodium salt will be used to activate RhoA. Unidirectional strain will be induced under both conditions and the effects of these agents on MRTF-A nuclear localisation and consequently αSMA expression will be investigated.

NANOSCALE MECHANOTRANSDUCTION: OSTEOGENESIS OF EMBRYONIC STEM CELLS AND DEVELOPMENT OF OSTEOBLAST MIGRATION *IN VITRO*

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In vitro differentiation of embryonic stem cells (ESCs) to the osteoblast lineage has been well established, typically using culture medium supplements (such as ascorbic acid, dexamethasone etc) to promote osteogenesis[1]. However, stimulation by nanoscale mechanotransduction using the 'Nanokick' bioreactor has been previously shown to promote osteogenesis of human mesenchymal stem cells[2].

We show the application of nanovibrations promotes osteogenesis of embryonic stem cells without the need for traditional osteogenic medium supplements. Our results show nanokicking causes mineralisation and expression of osteogenic markers RUNX2, osteocalcin and ALP in mESCs, at levels similar to calvarial osteoblasts (used as control). Interestingly nanokicking caused ESCs to form more numerous bone nodules with distinct morphology compared to those produced by osteogenic medium. The combination of nanokicking with standard osteogenic supplements also caused a synergistic increase in alkaline phosphatase activity, higher than either method alone.

Nanokicking also promotes chemotactic behaviour, a key function of osteoblasts during bone turnover and repair. As studied by an adherent chemotaxis model, osteoblasts response to PDGF-BB (a known osteoblast chemoattractant[3]) is increased following nanovibrational stimulation, revealing the influence of nanoscale mechanotransduction on the development of migratory behaviour in osteoblasts.

Nanovibrational osteogenesis provides a platform for simple, reproducible osteoblast culture without the need for expensive growth factors and supplements. The technique is scalable for regenerative medicine applications, using osteoblasts derived from readily expandable ESCs instead of patient derived cells and therefore reducing batch variability.

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BACTERIA ENGINEERING FOR EX-VIVO EXPANSION OF HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cell (HSC) survival and expansion in synthetic environments has been the aspiring goal of an increasing number of studies during the past decade. This rare population of cells are, except of major regulators of all stages of haematopoiesis, a potential solution for bone marrow transplantations in leukaemia, bone marrow failure or other haematological disorders. In the present study we aim to create an artificial 3D bone marrow-(BM) mimicking microenvironment based on non-pathogenic bacteria to support HSC proliferation. The system will consist of a biofilm, made up of genetically engineered populations of Lactococcus Lactis that will produce the soluble factors CXCL12, thrombopoietin (THPO) and Angiopoietin-1 (ANGPT1) as well as the adhesion molecules VCAM-1 and Stem Cell Factor (SCF) in a constitutive and inducible (light or nisin) manner. The aforementioned cytokines and adhesion proteins have been reported as vital for the proliferation of the HSCs in their natural microenvironment, amongst other factors like hypoxia and calcium concentration, that can be easily replicated as well. After the biofilm formation, CD34+ cells will be seeded inside a hydrogel cast on top of the biofilm, at a stiffness close to the one found in the BM. The hydrogel as well as the production of the cytokines by the bacteria will recreate a bone marrow microenvironment-like ex-vivo and provide the HSCs with the appropriate stimuli to maintain their phenotype and induce their expansion. We hypothesize that these BM niche factor expressing bacteria will provide a more simple and robust system for HSC growth than the use of cytokine cocktails or stromal cells reported in the literature.

ENGINEERING GROWTH FACTOR MICROENVIRONMENTS – A NEW THERAPEUTIC PARADIGM FOR REGENERATIVE MEDICINE

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The multi-centre EPSRC Programme Grant, "***Engineering growth factor microenvironments – A new therapeutic paradigm for regenerative medicine***" involves academics at the Universities of Glasgow, Nottingham, Imperial College London and the Scottish Blood Transfusion Service. The project focusses on developing three biomaterial mediated growth factor (GF) delivery methods and applying them in three therapeutic areas of need. In terms of bone repair there is significant need for osteoinductive bone graft material. Haematopoietic stem cells (HSCs) are regularly used for transplant (e.g. to repopulate/regenerate the blood system) however there is no viable method to expand HSC's ex vivo. iPSC derived cardiomyocytes can provide the basis for cardiotoxicity screening of drugs and perhaps to repair damage from myocardial infarction, however the immaturity of these cells is a barrier to use. In each of these areas we aim to utilise growth factors to tackle the present challenges.
The materials being developed include protein hydrogels, GF functionalised polymer coatings and enzyme/light controlled GF release. The aim being to deliver GFs in a controllable and efficient manner, unlike many existing clinical products involving supraphysiological doses of growth factors and associated complications. A seventh work package in this project is dedicated to the spatial measurement and tracking of growth factors based on our expertise in surface enhanced Raman spectroscopy.

IDENTIFICATION AND *IN VITRO* SCREENING OF OSTEOGENIC METABOLITES THROUGH SUPPLEMENT-FREE NANOVIBRATION-DRIVEN MESENCHYMAL STEM CELL DIFFERENTIATION

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Bone is one of the most commonly transplanted tissues but obtaining suitable autologous grafts is difficult and associated with donor site morbidity and pain, necessitating the development of effective tissue engineered approaches. In the laboratory, these approaches typically involve osteogenic differentiation of mesenchymal stem cells (MSCs) through media supplementation. We recently developed a supplement-free osteogenic differentiation protocol through nanovibrational-stimulation of MSCs; the 'nanokick' bioreactor. Here, we hypothesised that nanovibrational differentiation of MSCs would allow metabolomic analysis of differentiation without confounding exogenous media supplements. We aimed to investigate MSC nanovibration-driven osteogenesis in 2D and 3D cultures, identify key osteogenic metabolic processes and investigate their osteogenic potential by supplementing these pathways. The differentiation of MSCs cultured in standard 2D or 3D gel cultures over 28 days was assessed in three groups- nanovibrational stimulation, osteogenic media and MSC expansion media. Metabolomic analysis was performed (LC-MS; ZIC-pHILIC) to identify key metabolites in osteogenesis. We selected a promising metabolite, which was synthesised along with several other molecules with similar structures. Their osteogenic potential was investigated through gene and protein expression analysis. Nanovibration upregulated key osteogenic genes and proteins in both 2D and 3D cultures comparably to osteogenic media and consistent with progressive osteogenic differentiation, including early upregulation of RUNX2 (2D x14.5, $p<0.05$; 3D x11.5, $p<0.05$) followed by maturation marker osteopontin (2D x19, $p<0.05$; 3D x7.2, $p<0.05$). Corresponding increases in osteogenic proteins were also observed. Metabolomic analysis identified several important networks, with cholesterol sulphate (CS) identified as a promising metabolite target. CS and several derivatives were synthesised and when supplemented at 1 μM to cultures induced osteogenic gene and protein expression with similar efficiency to osteogenic media, while having less off-target effects. Nanovibration is a novel tool for the supplement-free study of MSC osteogenic differentiation. Here we have used the bioreactor to drive 2D and 3D osteogenesis studying comparing and contrasting 2D vs 3D differentiation. Further, we have used the bioreactor as a tool to identify metabolites that in themselves can induce osteogenesis and that can be modified to tune effect; that supplements were not needed for osteogenesis is critical in producing an artefact free small molecule background.

The authors would like to acknowledge the EPSRC for funding this project (EP/N013905/1).

USING SURFACE CHEMISTRY TO DESIGN AN IN VITRO HAEMATOPOIETIC STEM CELL NICHE

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The location of stem cells in the body and their surrounding environment modulate stem cell behaviour. In the bone marrow, mesenchymal stem cells (MSCs) reside in close proximity to hematopoietic stem cells (HSCs). It is understood that MSCs regulate the stemness and activity of HSCs by secreting regulatory cytokines such as CXCL-12, SCF, TPO and VCAM-1.

In this work, we use the unique surface chemistry associated with poly (ethyl acrylate) (PEA) to engineer an artificial extracellular matrix (ECM) model aimed at maximising cytokine expression in MSCs, in a bid to support maintenance and proliferation of HSCs. We have designed the model based around the ability of PEA to induce a network conformation of fibronectin (FN) when it adsorbed onto a spincoated PEA coverslip. This conformation allows cells to access both the integrin binding and growth factor binding domains of FN, which in turn induces synergistic signaling in MSCs when they are cultured in a monolayer on these surfaces. This model is a platform for incorporating a range of growth factors as well as a co-culture of MSCs and HSCs, and has been shown to retain the $CD34^{+}CD38^{-}$ stem cell phenotype of HSCs when they are cultured within models featuring PEA + FN.

Poster no 1

NANOVIBRATIONAL STIMULATION (NANOKICKING) FOR 3D OSTEOGENESIS IN BIPHASIC SCAFFOLDS; COMPOSITING OF FREEZE DRIED COLLAGEN SPONGE-HYDROXYAPATITE IN HYDROGELS FOR BONE TISSUE ENGINEERING

Wich Orapiriyakul[1], Penelope M. Tsimbouri[1], Peter Childs[2], Shaun Robertson[3], Paul Campsie[3], R.M. Dominic Meek[4], Richard O.C. Oreffo[5], K. Elizabeth Tanner[2], Manuel Salmerón-Sánchez[2], Stuart Reid[3], Matthew J Dalby[1].

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Nanovibrational stimulation (nanokicking) promotes osteoblastogenesis of human mesenchymal stem cells (MSCs) in 2D [1] and 3D [2]. We are developing biphasic scaffolds compositing collagen hydrogels and freeze dried collagen sponges to allow 3D culture within nanokick bioreactor aimed at clinical application.
To study the gel phase, Stro1 selected human MSCs seeded in 1.8 mg/ml rat tail collagen type I hydrogels containing 0%, 35% 58%, 70% dry weight hydroxyapatite (HA) were prepared. The mean elastic moduli of the HA-gels were 180, 194.6, 204.8, 182.8 Pa respectively as measured by rheology. At 1000 Hz frequency of nanovibrational stimulation, gel displacement amplitudes were consistency measured by interferometry at ~90 nm. Alamar blue and live-dead stains showed that the HA gels and nanovibration had no effect on cell viability. After 9 days of stimulation, a trend of osteogenic gene up-regulation (RUNX2, osteonectin, osterix) was observed. After 7 days of stimulation, western blotting showed phosphoRUNX2 vs total RUNX2 up-regulation in nanovibrated MSC scaffolds. A metabolomic study after 1 week of nanostimulation demonstrated involvement of lipid metabolism (energy), and predicted activation of ERK1/2 pathway and inflammatory metabolites highly suggestive that the nanovibrational technique enhanced osteogenesis through natural bone healing pathways.
To study the biphasic scaffold, 5% freeze dried collagen sponges were produced and integrated into MSC seeded gels. The average elastic modulus of dry sponges were 137.3 MPa (SD=71.61) measured by compression test and SEM showed the average pore size was 227.74 μm (SD 72.93). Interferometry showed good fidelity of nanovibrational stimulation for the biphasic scaffold. After 7 days of stimulation, gels were allowed to contract onto sponges; this is to use the stimulated cells natural contraction to stiffen the construct. At day 9, microscopy showed MSCs migrating from the gel into the sponge.
Nanovibrational stimulation in HA-hydrogels is safe for cells and, with nanovibrational stimulation, promotes 3D osteoblastogenesis. Biphasic collagen scaffolds allowed nanovibrational force transmission and improved composite handleability for clinical use. Biological effects of nanovibrational stimulation in biphasic scaffolds will be presented.

This work was funded by the Find a better way, BBSRC, EPSRC, Royal Thai Government.

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Poster no 2

ROLE OF IMPLANT NANOROUGHNESS AND BIOACTIVE COATING ON OSSEOINTEGRATION AND BACTERIAL GROWTH

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Introduction: Ti and its alloys can be processed to tune their physical and chemical properties. In this project, we are interested in the ability of a material to induce osseointegration and subsequent mineralization dependent on the initial adhesion of mesenchymal stem cells (MSCs) onto the implant surface. Further, we are interested in developing topographies that can kill bacteria. Polymers are also important medical materials. An example is a poly(ethylacrylate) (PEA) as it causes a spontaneous unravelling of fibronectin (FN) upon contact which facilitates interaction with growth factors (GFs), allowing ultra-low dose GF presentation with high efficiency. We thus aim to investigate the effect of Ti surfaces with bactericidal nanotopographies coated with PEA/FN/BMP2 to see if the coating can improve MSC growth and differentiation while maintaining bacterial kill. **Methods**: Two different Ti nanowire surfaces were produced through a thermal oxidation process under alkaline conditions (1h and 2h TiO_2, with average maximum height ~380- 550 nm respectively). Two different time points were used to coat the surfaces with PEA utilizing a plasma polymerization technique (90 seconds and 3 minutes). The biological coating was applied using FN/ BMP2 prior to Stro-1+ hBM-MSC culture. Physical and chemical characteristics were studied using SEM, AFM, WCA, and XPS. The availability of P5F3 (GF binding) and FN7.1 (Cell adhesion) domains was tested using antibody-based ELISA assays. **Results**: AFM showed that the maximum average height of 1h and 2h TiO_2 are *Rt* ~350 and ~700 nm respectively after 90 secs PEA coating and *Rt* ~390 and ~740 nm after 3 mins PEA coating. Polymer coating increased the hydrophobicity of Ti, which resulted in increased protein adsorption. On the other hand, FN decreased the hydrophobicity, which improved cell adhesion. The number of cell-binding domains increased on the coated surfaces compared to coated/ uncoated flat surfaces, and the heparin-binding domain increased on coated surfaces compared with uncoated. The current coating showed an improvement of cell growth, adhesion and osteogenic gene expression on TiO_2 nanowire surfaces. **Conclusion**: An ideal bone implant should enhance the osteogenesis and reduce bacterial adhesion. However, increasing the implant surface area, e.g. a 3D format could improve the osteogenic and bactericidal effect we seek.

Poster no 3

SHORT AND MIDTERM EFFECTS OF TOPICAL GLYCERYL TRINITRATE ON TENDINOPATHY: A SYSTEMATIC REVIEW OF RANDOMISED CONTROLLED TRIALS

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The limited existing evidence on the effectiveness of topical glyceryl trinitrate (GTN) on tendinopathy is controversial with published randomised controlled trials (RCTs) demonstrating conflicting results. Our aim was to assess the effects of topical GTN on the following parameters in all tendinopathies: a) pain, b) local tenderness, c) range of motion (ROM), d) tendon strength, e) patient satisfaction, e) absence of symptoms with activities of daily living (ADLs) and e) side effects.

A thorough literature search was conducted via Medline, EMBASE and Scopus aiming to identify RCTs comparing the effects of topical GTN with either placebo or other treatments on tendinopathy. Overall quality of each eligible study was determined based on a combined assessment of their internal validity, external validity and precision. The level of evidence for each assessed parameter was rated based on the system by Van Tulder et al. (1997) separately for short (0-8 weeks) and midterm (12-24 weeks) results.

A total of 10 eligible RCTs were identified including patients with tendinopathy of the rotator cuff (n=4), wrist extensors (n=3), Achilles (n=2), and patellar (n=1) tendons. For all tendinopathies, only improvements in pain (generic) were significant when comparing GTN Vs placebo in the short-term (Level 3 evidence). Significant improvements in mid-term outcomes for treatment with GTN vs placebo included the following: pain (generic, at night and with activity; Level 3 evidence); local tenderness (Level 3 evidence); ROM (Level 2 evidence); tendon strength (Level 2 evidence); patient satisfaction (Level 1 evidence); chances of being asymptomatic with ADLs (Level 1 evidence). Patients treated with topical GTN reported a higher incidence of headaches than those who received placebo (Level 2 evidence).

Treatment of tendinopathies with topical GTN for up to 6 months appears to be superior to placebo, however well-designed studies are warranted to provide insights into its long-term outcomes.

Poster no 4

MICROFLUIDIC FABRICATION OF MICRON-SIZED 3D ENVIRONMENT FOR STEM CELL DIFFERENTIATION INDUCED BY ENGINEERED BACTERIA

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In this study, we report a droplet-based microfluidic system for encapsulation of mesenchymal stem cells and non-pathogenic bacteria into micron-sized 3D hydrogel scaffold made from alginate. The bacteria, Lactococcus lactis, was engineered to express growth factors (BMP-2 and FN7-10) in presence of chemical stimuli therefore inducing the differentiation of the MSCs into osteoblast over the course of 2 weeks.

Poster no 5

CONTROLLING THE ENEMY: BACTERIAL BIOFILMS FOR MSC DIFFERENTIATION

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Abstract

Use of materials is attractive in stem cell cultures as surface area can be scaled up using 3D printing or fibrous hydrogels. Materials can be tuned to deliver specific, and even dynamic, biological cues to stem cells. However, they are still very limited as stem cells are regulated by a complex biological milieu.

We present a novel approach using simple cells, 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. Here we show that controlled expression of fibronectin fragments [1, 2] supports growth and temporal regulation of secreted bone morphogenetic protein 2 drives osteogenesis in an on-demand manner.

The fibronectin fragment allows mammalian cell adhesion through integrins and cells show the same behaviour as when seeded over fibronectin coated surfaces. Moreover, the direct contact of the mammalian cells with the bacteria allows for instantaneous delivery of the growth factor bone morphogenetic protein 2, a known and potent osteogenic inducer. The combination of these proteins has displayed stem cell differentiation in the short, mid and long term that is equivalent to that of 100 ng/mL of exogenous addition of bone morphogenetic protein 2; creating a new paradigm in surface engineering for regenerative medicine. The system also has the potential to express a variety of proteins in order to tackle a number of different therapeutic problems.

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Poster no 6

NANOPARTICLE LABELLING TO FACILITATE HIGH-RESOLUTION NON-DESTRUCTIVE IN VIVO 3D IMAGING OF PERIPHERAL NERVE REGENERATION.

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Peripheral nerve injuries are common (1/1,000 incidence in Europe) and have poor recovery due to insufficient understanding of the neurobiology of injury. A lack of non invasive assessment of nerve regeneration has hindered the translation of novel therapies. At the University of Glasgow and collaborating laboratories we have used adipose derived stem cells (ADSC) to augment peripheral nerve regeneration *in vitro* and *in vivo*. One of the challenges of this approach is detecting what happens to the cells in vivo following transplantation.

The ADSCs were harvested in keeping with the Human Tissue Act (2004) and biobank approval (BB170519) and labelled utilising green fluorescing (490nm emission), 200nm SPIONs (Chemicell) at a concentration of 0.01mg/ml and 0.1mg/ml. We evaluated the impact of nanoparticle dosing and viral multiplicity of infection on ADSC function using alamar blue and ELISA. The cells were traced and detected following transplantation using immunohistochemistry and acquired a 3D rendered image using micro computerised tomography (μCT) scanning, with a resolution better than 5μm. Analysis of conduit density and nerve regeneration was performed on CTVox, CTan and FIJI software.

SPION labelling was not associated with cell death and was successfully used to trace and detect cells following transplantation *in vivo* using μCT. 98% of human ADSCs exhibited uptake of nanoparticles. Alamar Blue analysis indicated that SPIONs increase ADSC proliferation over time (no nano vs 0.01 day 5 $p=0.01$, Mann Whitney $n=3$).

This study demonstrates that SPION contrast enhancement facilitates the detection of therapeutic stem cells and further evidences the potential for high resolution non-invasive imaging to improve patient outcomes following peripheral nerve injury.

Poster no 7

MICROFLUIDIC COLLAGEN HYDROGELS FOR BMP-2 DELIVERY

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During recent years, microgels have emerged as an effective drug delivery system (DDS), showing advantages such as tuneable size, increased surface area and injectability [1]. Collagen has been extensively studied as a scaffold for regenerative medicine and drug delivery due to its biocompatibility, non-immunogenicity and degradability[2,]. In this study, we show the use of microfluidic techniques for the automated generation of monodisperse type-I collagen (col-I) microgels and the encapsulation of hollow collagen spheres loaded with BMP-2 for regenerative therapies in bone repair.

Microgels were synthetized in a glass microfluidic device with co-flow configuration. Crosslinking occurs after merging col-I with the PEG-4S crosslinker in the nozzle of a double-chamber capillary within an oil flow. A coiled tube is placed in the outlet in order to increase the residence time of the microparticles, allowing them to gelify inside the microfluidic system. The chemistry, stiffness and degradation of the synthesised col-I microgels will be characterized and the viability of osteocytes assessed. Previous work on astrocytes has shown low cytotoxicity after 2 and 5 days. Hollow spheres (diameter ~ 200 nm) are prepared by covalently binding collagen on silica templates, which are subsequently removed with hydrofluoric acid. Hollow spheres can be loaded with BMP-2 or other relevant growth factors by diffusion, and encapsulated in the microgels within the microfluidic device.

We demonstrate that microfluidics is an adequate technique for automatically generating monodisperse collagen microgels and provides a useful tool for the posterior encapsulation of nanospheres and cells. The microgels are non-cytotoxic to cells and foster cell growth at different conditions. Microgels encapsulating hollow spheres can provide sustained delivery of different therapeutic factors as found in literature [3].

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Poster no 8

THE SYNOVIAL SECRETOME CONTRIBUTES TO CARTILAGE PATHOLOGY IN OSTEOARTHRITIS: A ROLE FOR EXOSOMES

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In addition to classical cartilage damage, seventy percent of osteoarthritis (OA) patients present with synovial inflammation. This results in an altered secretory profile from the synovial tissue that includes microvesicles and exosomes. Exosomes carry and transfer cargo, such as proteins, lipids and RNA, which can have regulatory functions. Thus, the impact of isolated OA synovial exosomes on primary OA articular chondrocytes was evaluated.

Human OA synovial explants (n=35 collected by arthroplasty) were cultured for 48h in the presence/absence of IL-1β, PAR2 agonist peptide (SLIGKV-NH_2) or a reverse peptide (RP), and conditioned media (CM) prepared. Dead cells and debris were eliminated by differential centrifugation from CM, and exosomes isolated by ultracentrifuge (100,000g). Nanoparticle tracking analysis (NTA), scanning/transmission electron microscopy (SEM/TEM) and western blot (WB) analysis was used to evaluate exosome preparations. Exosome content (RNA and protein) was fluorescently labelled and uptake by chondrocytes (n=6) evaluated. Chondrocyte (n=4) gene expression post-exosome uptake was analysed by qPCR. Levels of inflammatory cytokines and matrix metalloproteinases (MMPs) were evaluated by ELISA.

Western blots confirmed presence of exosome-associated markers CD9, CD81, HSP70 and CD63. NTA and SEM data established that 80-90% of microvesicles were the correct size, (30 to 150 nm) (Figure 1). Levels of IL-6, TNF-α and MMP-3 in explant derived CM were significantly increased by IL-1β and SLIGKV-NH_2 ($p<0.001$), compared to controls. SLIGKV-NH_2 also significantly increased IL-8 ($p<0.001$). Exosomes were taken up by chondrocytes 4 hours post-exposure, as determined by protein and RNA transfer. Human chondrocytes cultured in media containing IL-1β- and SLIGKV-NH_2- stimulated, synovial membrane-derived exosomes showed significant decreases in COL2A1 ($p<0.001$) and ACAN expression ($p<0.001$).

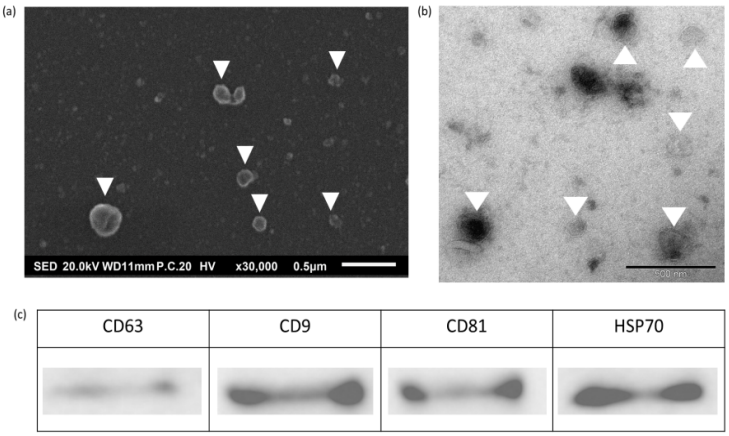

Figure 1: Characterisation of exosomes with (a) SEM (b) TEM and (c) WB

Conclusion. OA synovial tissue potentially impacts chondrocyte behaviour through the release of regulatory exosomes. Further characterisation of exosome cargo will provide insight into the role of the synovium in OA cartilage pathology.

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Poster no 9

BIOMIMETIC FUNCTIONALISATION OF BIODEGRADABLE PLLA WITH ACRYLATE BRUSHES

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Poly L-Lactic acid (PLLA) has been used as a biodegradable polymer for many years; key characteristics of this polymer make it a versatile and useful resource for regenerative medicine. One obstacle in utilising PLLA as a cellular environment for implantation is poor cellular adhesion due to its inefficient adsorption and expression of key biological signals. Here we show chemical modify of PLLA surfaces with poly (ethyl acrylate) (PEA) brushes able to induce the organisation of the extracellular matrix component, fibronectin (FN), into physiological-like fibrils. These FN fibrils expose binding motifs critical for cell adhesion and differentiation, including domains for the binding of growth factors (GFs).

The characteristic properties (stiffness, topology and biodegradation) of PLLA are ideal for regeneration applications in vivo, allowing stem cells to specialize within an implanted scaffold and eventually transfer the mechanical stress to the engineered tissue. We have established a protocol to polymerize PEA brushes on PLLA while maintaining the bulk properties of this polymer by using an activator regenerated electron transfer (ARGET) surface-initiated atomic transfer radical polymerisation (SI-ATRP) technique to achieve a thin molecular coating of PEA. Beside surface characterization via AFM, XPS and WCA to optimize PEA grafting, we investigated the biological activity of these surface modifications in terms of fibronectin adsorption and cell response. PEA brushes triggered FN organisation into fibrils, which retained their ability to specifically bind growth factors. Cell adhesion and differentiation studies confirmed the potential of PEA brushes to create a more favourable and controlled microenvironments via surface modification of a biodegradable polymer.

Poster no 10

A NOVEL DUAL INJURY OSTEOARTHRITIS (OA) MODEL THAT COMBINES DESTABILISATION OF THE MEDIAL MENISCUS AND CARTILAGE DAMAGE LEADS TO ACCELERATED OSTEOPHYTOGENESIS.

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Osteoarthritis is associated with articular cartilage damage. While it is not knownhow disease pathogenesis is initiated, it is assumed that it can be via an isolated injury, or a combination of joint damage over time. Given the fact cartilage has poor regenerative capability, OA represents a major clinical challenge. Several murine models are used to study OA (e.g. destabilisation of the medial meniscus (DMM)), however do not combine simultaneous cartilage injury with joint destabilisation. The principle aim of this study was to investigate if combining cartilage damage and DMM accelerates the onset of OA-like symptoms.

OA was induced in mice via (a) transection of the medial meniscotibial ligament (DMM), (b) microblade scratches of articular cartilage (cartilage damage) or (c) combined DMM and cartilage scratch (DCS). Fourteen days post-surgery, dynamic weight bearing was assessed as an indirect measurement of pain, and microcomputered tomography (μCT) was used to monitor bone changes.

Osteophytes were present in all groups post-surgery. However, between groups there were observable differences in number, appearance, and bone volume (Fig. 1). Osteophytes in the DCS model encompassed a larger area of subchondral bone compared with DMM or cartilage scratch models (14.12 ±0.31 versus 12.4 ±0.62, 12.68 ±0.54, $p<0.01$ respectively). In DMM 6/8 mice developed protruding osteophytes with an arboreal-like structure. Osteophytes were present in all mice that underwent cartilage scratch surgery, and had a larger more calcified appearance. In the DCS model, all mice exhibited ≥2 large, protruding, calcified osteophytes. Finally, mice in the DCS model demonstrated a significant increase in front paw load compared with the other models, suggesting an increase in OA-like pain.

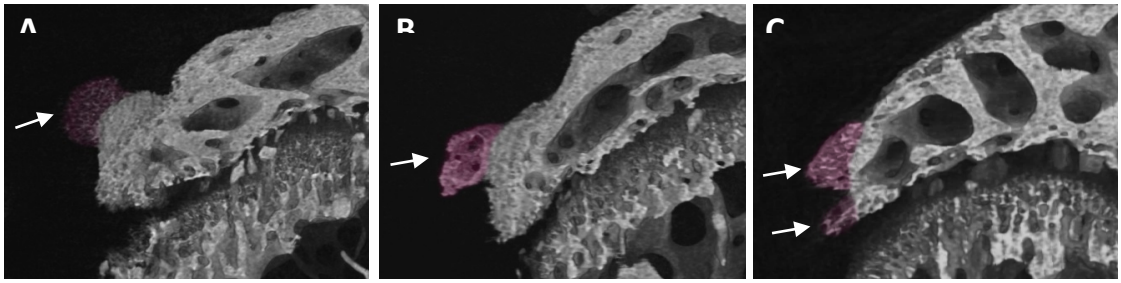

Figure 1. Osteophytes differ in number, appearance and bone volume between groups. (A) DMM model exhibits and arboreal osteophyte structure. **(B)** Cartilage scratch models display osteophytes with a larger, more calcified appearance. **(C)** DCS models present with ≥2 large, protruding, calcified osteophytes.

Conclusion: Combining DMM with cartilage damage provides a robust and reproducible model for OA-associated osteophytogenesis. This model also incorporates OA-related pain, arguably one of the most problematic and physically limiting symptoms of OA.