



July 2nd – 4th, 2014
Newcastle University



**The 2014 Annual Tissue & Cell Engineering
Society Meeting**
**(with UK Regenerative Medicine Platform
Hubs: Joint Annual Science and Industry
Meeting)**

Conference Book

**Organisers: Oana Bretcanu, Chaozong Liu
Helen Kelt, Fabio D'Agostino**

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Message From TCES President



I would like to welcome you, the TCES community, to the 2014, 16th annual conference in Newcastle.

COMMUNITY is a metaphorically big word, loaded as it is with high aspiration. But I think that we can now justifiably claim to be a true community, through the level of cohesion, stability and common interest that we enjoy.

Another defining characteristic of a community is inclusivity. Certainly over the last 5 years TCES can claim a major success in drawing in new partners, from a wider range of institutions and universities, who have hosted our conferences. Newcastle University boasts an impressive range of tissue engineering and regenerative medicine activities from engineering to stem cells, via musculoskeletal disease and biomechanics. This first TCES visit to Newcastle will doubtless show us some great science in detail, and hopefully means that the community grows a little more diverse and representative.

Rather like a mini-reflection of this wider TCES community, there is now also a healthy, integrated TCES committee at its core. This encourages and enables support of its young researcher community. I have watched an ever increasing stream of early-morning bright ideas and late-night efforts to develop schemes, prizes, experiences and responsibilities which will help the careers of its members: CV-fodder and genuine opportunities for initiative. At the other end of this scale, TCES is now able to use its networking strengths to help and to inform public bodies and established research consortia. At the Newcastle meeting you will be able to meet and see work from the Research Council Hubs in Regenerative Medicine. This is a key piece of two directional networking – members of the hubs get to understand (and perhaps integrate with) excellent science across our community. At the same time, you can get to show off your skills and ideas to these leading centres – who knows, you may be just what they are looking for as their programmes develop.

On a personal note, this will be my last conference as TCES president. The years since 2009 when I have helped to guide TCES along its development have been amongst the most rewarding I can remember. From the efforts of its members and committee, TCES has established a shape, cohesion and direction. It now needs a fresh mind to capitalise on these strengths. But this is not an onerous task, given that the TCES has such a contagious aim and engaging ethos, towards promotion of its best up-coming talent.

So I hope you will not only learn about some outstanding science and engineering in Newcastle, but also some of the secret values of networking, collaboration and friendship, within your community.

I wish you a remarkable conference.

A handwritten signature in black ink that reads "R Brown". The signature is written in a cursive, flowing style with a long horizontal stroke underneath the name.

Robert A. Brown.
President of TCES.

TCES2014 Organisers' Welcome

It is a great pleasure to welcome you to the 2014 Annual Tissue & Cell Engineering Meeting with UK RM Platform Hubs: Joint Annual Science and Industry Meeting!

We have received more than 130 abstracts, over half of the abstracts are authored by young and early stage researchers. This indicates the TCES is continuing thrive and has strong vitality! We would like to thank every member of the TCES community and all delegates attending the meeting. Your enthusiastic participation ensures the very success of the TCES2014.

This TCES meeting has an exciting 3 days programme addressing stimulating and relevant areas of tissue and cell engineering in eight scientific sessions. We hope this meeting will provide an interesting arena and give you the opportunity to hear what's happening in the field, exchange ideas and networking with colleagues.

We hope its enjoyable social events will complement the exciting scientific programme at this stage in the evolution of TCES.

Best wishes to all delegates,

Oana Bretcanu, Chaozong Liu, Helen Kelt, Fabio D'Agostino

General Information

Conference Venue:

Stephenson Building, School of Mechanical and Systems Engineering, Newcastle University

Conference Room: Room F16,

Venue for Lunch/Coffee, Posters and Exhibitions: Room T12/T13,

Reception & Ceilidh dance: Civic Centre

Address: Barras Bridge, Newcastle upon Tyne NE1 7RS

Newcastle Civic Centre is a local government building located in the Haymarket area of Newcastle upon Tyne, England. It is the main administrative and ceremonial centre for Newcastle City Council.

Conference Dinner: [Thistle Hotel](#),

Neville Street Newcastle NE1 5DF,

Tel: 0871 3769029

Thistle Hotel Newcastle directly opposite the Newcastle Train Station and Metro stations.

Transport to the station or airport:

If you would like assistance with arranging transport after the meeting, please let the organizers know on the morning of Friday 4th July, or before if you need to leave early.

Local dialect: Geordie

Translation service available through the organisers.

Invited Speakers



Professor Kenny Dalgarno is Sir James Woodeson Professor of Manufacturing Engineering at Newcastle University. For the past decade he has been researching in the area of additive manufacture, with applications in rapid prototyping and manufacturing; polymer engineering; and with an increasing emphasis on applications in biomedical engineering, tissue engineering, and regenerative medicine, with work supported by the EPSRC, the European Commission, Arthritis Research UK, the Carbon Trust, and industry



Professor Zhanfeng Cui is the Donald Pollock Professor of Chemical Engineering, University of Oxford and the founding Director of the Oxford Centre for Tissue Engineering and Bioprocessing. His current research interests include bioreactor technology for tissue culture and stem cell expansion, cryopreservation of stem cells and engineered tissue, tissue engineering monitoring and stem cells for drug and toxicology testing.



Professor Williams is University Academic Lead for Health and Wellbeing at Loughborough University and leads the national £5.3m EPSRC Centre for Innovative Manufacturing in Regenerative Medicine. Between 1999 and 2003 David was Technical Director of Bepak, a major supplier of drug delivery devices to the pharmaceutical industry. This followed ten years as Professor of Manufacturing Processes at Loughborough. His early career, while including industrial jobs with GKN and Metal Box, was primarily within the Cambridge University Engineering Department. David was elected a Fellow of the Royal Academy of Engineering in 2002. He is a member of the Department of Health Regenerative Medicine Expert Group.



Professor Cosimo De Bari is a clinically active rheumatologist with expertise in regenerative medicine for musculoskeletal applications.

Cosimo graduated in Medicine (*maxima cum laude*) from the University of Bari (Italy), where he also underwent specialist training in Rheumatology. He then moved to Belgium, where he obtained his PhD from the University of Leuven and was recipient of the Rotary Young Investigator Award 2003 from the Royal Belgian

Society for Rheumatology.

In 2003 Cosimo moved to the UK in the Department of Rheumatology at King's College London. In May 2005 he was awarded a Clinician Scientist Fellowship from the Medical Research Council and in December 2005 he was appointed Clinical Senior Lecturer & Consultant Rheumatologist.

Since September 2007 Cosimo is Professor of Translational Medicine at the University of Aberdeen, where he heads the Regenerative Medicine Group in the Musculoskeletal Research Programme.

Cosimo has expertise in stem cell research for musculoskeletal repair, regenerative medicine and tissue engineering. Current research interests in his Aberdeen Regenerative Medicine Group include (i) the development of stem cell-based tissue engineering products for cartilage and bone repair; (ii) the study of the stem cell niches in the joint in health and diseases such as osteoarthritis and rheumatoid arthritis; (iii) the investigation of the developmental ontogeny of mesenchymal stem cells.



Prof. Lako completed her BSc degree in Biochemistry/Genetics at Tirana University in Albania. Her motivation to further study Human Genetics was realised when she joined Profs. Strachan and Lindsay's groups at Newcastle University to

complete PhD studies in the field of human developmental genetics. Since 2003, Prof. Lako runs her own independent research group with particular focus on (i) the understanding of self-renewal in human pluripotent stem cells; (ii) generation of functional and transplantable blood, corneal and retinal cells from human pluripotent stem cells and (iii) clinical translations of basic biology studies to treat corneal and retinal blindness.



Fergal O'Brien currently heads one of the largest regenerative medicine research groups in Ireland. He is a graduate in mechanical engineering from Trinity College, Dublin. His PhD research was in the area of bone mechanobiology (awarded from TCD in 2001). He subsequently carried out postdoctoral research in orthopaedic tissue engineering at Massachusetts Institute of Technology in collaboration with Harvard Medical School before his appointment, in 2003, as a Lecturer in Anatomy in RCSI. He was promoted to Senior Lecturer (2006) and Associate Professor (2007). In addition he holds an adjunct appointment as Associate Professor in Bioengineering in TCD and is a PI and steering committee member of the Trinity Centre for Bioengineering. His research focuses on bone mechanobiology and osteoporosis and the development of novel scaffolds for tissue engineering. He has a specific interest in the application of stem cell biology and gene therapy to these scaffolds and the biomechanical factors which control stem cell differentiation.

He has been awarded a number of scientific honours including a Fulbright Scholarship (2001), New Investigator Recognition Award by the [Orthopaedic Research Society](#) (2002), Science Foundation Ireland, [President of Ireland Young Researcher Award](#) (2004), [Engineers Ireland](#) Chartered Engineer of the Year (2005) and most recently, in 2009, a [European Research Council Investigator Award](#) (2 million). He is a reviewer for over 30 journals and a grant reviewer for agencies in Europe (FP7), UK, Australia, USA and Ireland. He is currently Treasurer of the Biomedical Division of Engineers Ireland, Editorial Consultant for the *Journal of Biomechanics* and Associate Editor (Tissue Engineering) for the [Journal of the Mechanical Behavior of Biomedical Materials](#).



Dr Frances Henson is a Senior Lecturer in the Department of Veterinary Medicine at the University of Cambridge. Her research focuses on the mechanisms underlying osteochondral and chondral repair, regeneration and remodelling. Current research projects include the effects of tissue engineering and biological factors on osteochondral and chondral healing, development of a bio-compatible meniscal scaffold and the response of bone to high intensity exercise.

Dr James Phillips is a Senior Lecturer in Biomaterials & Tissue Engineering at University College London. His group specialises in nervous system tissue engineering, in particular building artificial nervous system tissue for regenerative medicine, or for use as 3D co-culture models in neuroscience research (www.jamesphillips.org). His first degree was in Biochemistry at Imperial College London, followed by a PhD in Pharmacology at the School of Pharmacy, University of London, awarded in 2000. He was a postdoctoral researcher on an EU project in the Tissue Repair and Engineering Centre at UCL, then worked as a Research Fellow in the Surgery Department at UCL. From 2004 to 2013 he was a Lecturer in Health Sciences at the Open University, involved in teaching, research and public engagement activities. James is currently involved in a range of research projects including collaborations with scientists, clinicians and engineers working in academia, industry and healthcare. He is a member of the Editorial Board for the *Journal of Biomaterials Applications* and is part of the Executive Committee of the Tissue and Cell Engineering Society.

Conference Scientific Programme

Wednesday, 2nd July 2014

Time	Event	Venue
10:00-17:30	Registration & posters setup	T12/T13
12:30-13:30	Lunch	T12/T13
13:30-14:00	Welcome and opening remarks	F16
14:00-15:45	Session 1: Clinical translations Chair: Prof Andrew McCaskie & Dr Fran Henson	F16
14:00-14:30	Seeing through stem cells Prof Majlinda Lako, University of Newcastle	F16
14:30	The use of large animal models in the pipeline from bench to clinic Frances Henson, University of Cambridge	F16
14:45	Engineering of a Functional Tendon Using Load bearing suture technique and Tissue Engineered Collagen Graft: An in vivo Study Prasad Sawadkar, University College London	F16
15:00	Development of an Acellular Xenogeneic Nerve Graft Leyla Zilic, University of Leeds	F16
15:15	Living biointerfaces to direct cell function Manuel Salmeron-Sanchez, University of Glasgow	F16
15:30	The Effect of Long-term 3D Culture on Cell Morphology and Behaviour Alisha Chhatwal, Durham University	F16
15:45-16:15	Coffee break and posters	T12/T13
16:15-18:00	Session 2: Stem cells Chair: Prof Cosimo De Bari & Amanda Barnes	F16
16:15-16:45	Mesenchymal stem cell niches in joint health and disease Prof Cosimo De Bari, University of Aberdeen	F16
16:45	Dynamic Surfaces for Modulating Stem Cell Fate Laura E. McNamara, University of Glasgow	F16
17:00	Characterisation of distinct regional and immuno-selected cell populations derived from human foetal femurs and their capacity for bone defect repair and regeneration David Gothard, University of Southampton	F16
17:15	Mesenchymal Stromal Cells Organise Endothelial Networks through PDGF, ILK and FGF Signalling Julia Marshall, University of Leeds	F16
17:30	Modulation of Human Skeletal Stem Cell Fate and Function as a Consequence of Nanotopography and Hypoxia Emma S McMorrow, Southampton University	F16
17:45	The "Cryptic" Extracellular Matrix and Its Impact on Stem Cell Differentiation Christine-Maria Horejs, Imperial College London	F16
19:00	Reception and Ceilidh dance	Civic Centre

Thursday, 3rd July 2014

Time	Event	Venue
8:30-10:30	Registration & posters setup	T12/T13
9:00-10:45	Session 3: Cell biology Chair: Prof Vivek Mudera & Josephine Wong	F16
9:00-9:30	Nerve Tissue Engineering Dr James Phillips Biomaterials & Tissue Engineering, University College London	F16
09:30	Engineering the corneal stroma: The effect of a three-dimensional environment and growth factor supplementation Laura Sidney, University of Nottingham	F16
09:45	Hypermethylation in OA - Transactivation of the COL9A1 proximal promoter region by SOX9 in chondrocytes is regulated by DNA methylation Maria C de Andres, University of Southampton	F16
10:00	Photochemical Functionalisation and Direct-Write Patterning of Diamond-Like-Carbon for Neural Interfaces James Dugan, University of Sheffield	F16
10:15	Influencing the Skeletal Stem Cell Niche by Targeted Liposomal Wnt Delivery Agnieszka A Janeczek, Southampton University	F16
10:30	Clay gels localise and enhance BMP2 induction of osteogenesis Jon Dawson, University of Southampton	F16
10:45-11:15	Coffee break and posters	T12/T13
11:15-12:30	Session 4: Bioreactors Chair: Prof Zhanfeng Cui & Richard Balint	F16
11:15-11:45	Engineering Human: 3D Neural Network – An Interdisciplinary Approach Keynote: Prof Zhanfeng Cui University of Oxford	F16
11:45	Nano-Kicking stem cells into making Bone Gabriel D. Pemberton, University of Glasgow	F16
12:00	Adapting aligned, stabilised 3D tissues for large-scale neurobiological research Caitriona O'Rourke, Open University	F16
12:15	Real time spatial monitoring system for 3D culture models Dominique Thomas, ¹Eastman Dental Institute, University College London	F16
12:30	Development of a novel biorheometer for in vitro real time monitoring of matrix remodelling Adam Glen, Cardiff University	F16
12:30-14:00	Lunch and posters	T12/T13
13:00-14:00	TCES AGM	F16
14:00-15:45	Session 5: Musculoskeletal tissue engineering Chair: Prof Kenny Dalgarno & Dr. Paul Genever	F16
14:00-14:30	Novel materials and biofabrication strategies for musculoskeletal tissue engineering Keynote: Prof Kenny Dalgarno Newcastle University	F16

14:30	Identification of distinct niches of functional mesenchymal stem cells in bone marrow and synovial tissues in a mouse model of joint surface injury (Angela R. Armiento)	F16
14:45	EGF and PDGF elicit Wnt-independent β -catenin signalling in mesenchymal stromal cells via integrin-linked kinase Charlotte Knight, University of York	F16
15:00	Synthesis and In Vitro Biocompatibility of Multi-substituted Hydroxyapatite for Bone Tissue Engineering Applications (Yanny M. Baba Ismail, Keele University)	F16
15:15	Enhanced Osteogenesis of hMSC in Hydroxyapatite/Fibrin Gels Simon Partridge, Newcastle University	F16
15:30	Electrostatic Stabilisation of Bio-Ink through the Cationic Encapsulation of Cells for Piezo Drop on Demand Inkjet Printing Matthew Benning, Newcastle University	F16
15:45-16:15	Coffee break and posters	T12/T13
16:15-17:45	Session 6: Tissue engineering scaffolds Chair: Prof F O'Brien & Fabio D'Agostino	F16
16:15-16:45	Collagen-based scaffolds for controlled delivery of genes & growth factor in tissue engineering Keynote: Prof Fergal J. O'Brien Royal College of Surgeons in Ireland	F16
16:45	Control of Collagen Hydrogel Compression for Cell Rescue Josephine Wong, University College London	F16
17:00	Cell and Bone Donor age Influence the Osteogenic Activity of MSCs Cultured on an Osteoinductive Washed Human Bone Scaffold Christopher A Smith, The University of Manchester	F16
17:15	Hydrogels from demineralized and decellularized bone extracellular matrix for bone regeneration Lisa J White, Nottingham University	F16
17:30	Investigation of potential osteogenesis influence of carbon based nanomaterials Hatice Kose, Keele University	F16
19:00	Dinner	Thistle Hotel

Thursday 3rd July 2014

UKRMPH sessions (closed sessions)

Time	Event	Venue
10:00-12:30	<p style="text-align: center;">Internal Hub Review (parallel sessions; each Hub will meet independently to review progress)</p> <p style="text-align: center;">Acellular Technologies Hub</p> <p style="text-align: center;">Safety and Imaging Hub</p> <p style="text-align: center;">Stem Cell Niche Hub</p> <p style="text-align: center;">Cell Behaviour, Differentiation and Manufacturing Hub</p>	<p style="text-align: center;">T1</p> <p style="text-align: center;">T10</p> <p style="text-align: center;">F1</p> <p style="text-align: center;">M9</p>
12:30-13:30	Lunch	T12/T13
13:30-15:30	<p style="text-align: center;">Hub to Hub Interactions (parallel sessions open to Hub members, industrial members and invited academics)</p> <p style="text-align: center;">Session 1: Extracellular Matrix Lead: Cay Kielty</p> <p style="text-align: center;">Session 2: Liver and Kidney Lead: Stuart Forbes and Adrian Woolf</p> <p style="text-align: center;">Session 3: Quality, Manufacturing and Regulatory Issues Lead: Peter Andrews/David Williams</p>	<p style="text-align: center;">T1</p> <p style="text-align: center;">T10</p> <p style="text-align: center;">F1</p>
15:30-17:30	<p style="text-align: center;">Potential New Collaborators (projects presentations from groups outside the Hubs)</p> <p style="text-align: center;">Session 1: Acellular Technologies Focus</p> <p style="text-align: center;">Session 2: Stem Cell Niche Focus</p>	<p style="text-align: center;">T1</p> <p style="text-align: center;">T10</p>

Friday, 4th July 2014

Time	Event	Venue
8:30-10:30	Registration & posters setup	T12/T13
9:00-10:45	Session 7: Cellular Therapies Chair: Prof Anne Dickinson & Prof Francisco Figueiredo	F16
9:00	Introduction to Cellular Therapies in Newcastle (Anne Dickinson)	F16
9:10	Limbal Stem Cell Deficiency – Bridging the Gap for Sight (Francisco Figueiredo)	F16
9:30	ASCOT - The trials and tribulations of setting up a clinical trial (Sally Roberts)	F16
9:50	Athersys Inc - Commercialization Challenges in Cell Therapy for Acute Ischemic Stroke (Robert Deans)	F16
10:10	Autologous Tolerogenic Dendritic Cells in Rheumatoid and Inflammatory Arthritis (Gillian Bell)	F16
10:30	Development of a Manufacturing Strategy for ReNeuron's CTX Cellular Therapy Program (Gary Brooke)	F16
10:45-11:15	Coffee break and posters	T12/T13
11:15-13:00	Session 8: UKRMP and other National Initiatives Chair: Professor Kevin Shakesheff	F16
11:15	Safety and Efficacy Hub (Trish Murray)	F16
11:30	Acellular Hub (Kevin Shakesheff)	F16
11:45	Cell Behaviour, Differentiation and Manufacturing Hub (Peter Andrews)	F16
12:00	Stem Cell Niche Hub (Stuart Forbes)	F16
12:15	Update from the Technology Strategy Board (Mike Sullivan)	F16
12:30	EPSRC Centre for Innovative Manufacturing in Regenerative Medicine (David Williams)	F16
12:45	Standardisation in Regenerative Medicine (Glyn Stacey)	F16
13:00-13:30	Closing remarks and awards	F16
13:30-14:30	Lunch	T12/T13

Abstracts for Oral Presentations

The use of large animal models in the pipeline from bench to clinic

Frances Henson

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The development of tissue engineering solutions for the repair and regeneration of tissues and organs is a fast moving area of scientific research. However, whilst the development stage of products is relatively straightforward, evaluation of the products produced is not. In this presentation I will discuss the use of large animal models in the development of successful tissue engineering solutions to musculo-skeletal problems.

The bio-compatibility of tissue engineering products is usually evaluated by simple *in vitro* cytotoxicity experiments. These experiments ensure that the materials are capable of supporting cell growth and that at least some of the basic functions of the cell, assessed by the production of key proteins and genes, are preserved. Small animal experiments are then often used to test *in vivo* safety.

The appropriate use of large animal models in musculo-skeletal product development is required for a number of reasons. These include the belief that the rigorous testing of a product in a biologically relevant environment is highly desirable prior to initiating clinical trials. In addition many tissue engineering products are classified as 'Advanced Therapy Medicinal Products' by the European Medicines Agency, and, as such, their licensing includes mandatory regulatory steps that include large animal model trials.

The choice of which large animal to use depends on a number of factors, including the country in which the study is performed, the finances available and the expertise of the group performing the work. For evaluation of chondral and osteochondral tissue repair, commonly used species worldwide include goats, sheep and horses.

Key to the use of large animal models for evaluation of tissue engineering constructs is an appropriate set of agreed clinical output data. This data should include functional evaluation of tissue repair, for example gait analysis and measurements of stiffness of repaired tissue. Once tissue is harvested, industry standard scoring systems, such as the International Cartilage Repair Society Gross Morphology score and the Modified O'Driscoll score should be used to quantitatively assess the quality of the repair tissue. Further assessments can be made by performing immunohistochemical analysis on histological sections. The identification of types I and II collagen is commonly investigated in chondral and osteochondral healing.

The use of an appropriate package of assessment tools for the evaluation of tissue engineering constructs allows the researcher to make an informed judgement on the suitability of a tissue engineering construct in the joint niche. This judgement will inform whether or not the construct proceeds to clinical trials.

Engineering of a Functional Tendon Using Load bearing suture technique and Tissue Engineered Collagen Graft: An *in vivo* Study

Prasad Sawadkar^{1*}, Paul Sibbons², Laurent Bozec³ and Vivek Mudera¹

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INTRODUCTION:

Every year in the UK over half million people suffer from tendon related disorder. Surgical management of the ruptured tendon is challenging because treated tendon often retracts and forms a gap. Presently these gaps are filled with auto-, allo- or, synthetic grafts but they all have clinical limitations. To overcome this problem, we have fabricated and tested tendon grafts *in vivo* which were made up of type 1 collagen which is natural polymer and predominant protein in the tendon.

METHODS:

Surgical procedure was carried out under ethical approval and home office licence. Thirty NZW rabbits weighing between 3-4.5 kg were used in this study. Acellular and cellular tissue engineered collagen grafts were sutured as previously described by Sawadkar et al. (1)

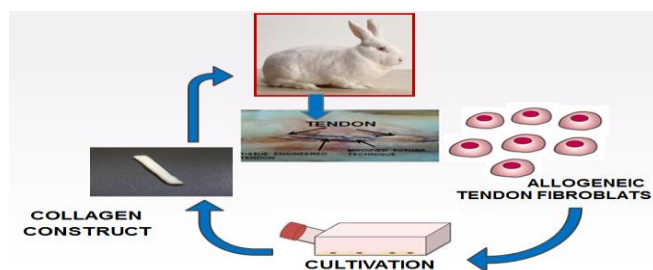


Figure 1 Showing an *in vivo* experimental set up

We have previously reported that this technique partially loads constructs and shown that this load up-regulates tendon remodelling genes *in vitro*. Mechanical tests were performed after 1,3 and 12 weeks along with histological and microscopic analysis of the grafts.

RESULTS:

Gross observation at 3 and 12 weeks showed bridged integration of the graft without any adhesion (Fig.1) with significant increase in the mechanical properties [35.02 ± 2.1 N ($p < 0.05$) for 12 weeks and [16.26 ± 0.58 N ($p < 0.05$)] for 3 weeks as compared to 1 week (5.19 ± 0.14 N).

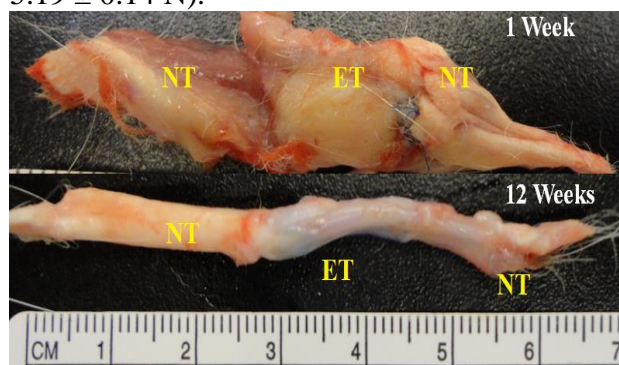


Figure 2 Showing tissue engineered collagen constructs (ET) incorporated in native tendon (NT)

Histological analysis showed that tendon fibroblasts from the native tendon were able to migrate to the graft with higher collagen remodelling and graft maturation at 12 weeks as compared to 3 and 1 week.

DISCUSSION & CONCLUSIONS:

Insertion of tissue engineered collagen graft using a novel load bearing suture technique which partially loads the construct *in vivo* showed integration, greater mechanical strength and no adhesion formation in the time period tested

REFERENCES:

1. Sawadkar P, Alexander S, Tolk M, Wong J, McGrouther D, Bozec L, et al. (2014). *BioResearch* open access. ;2(5):327-35

ACKNOWLEDGMENTS:

Authors would like to thank government of India for funding this project.

Development of an Acellular Xenogeneic Nerve Graft

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Introduction

Peripheral nerve injuries affect 1 in 1000 of the population [1]. Injury gaps greater than 1-2cm are bridged using autografts which direct regenerating axons by topographic guidance [2]. Commercially available products are not particularly suitable as they lack architecture similar to that of the native ECM of the nerve. An acellular nerve would address regeneration of axons at the cellular level, encouraging regeneration within a native guidance environment. The present aim is therefore to develop compatible, non-immunogenic, nerve grafts to restore sensory and motor function using a novel technique to decellularise porcine nerve. It will then be used as a scaffold for seeding primary Schwann cells in comparison with a graft alone for early stage evaluation.

Materials and Methods

The sciatic, tibial, common peroneal and sural nerves are under investigation as potential conduits. The nerves were decellularised using low concentration sodium dodecyl sulphate and nuclease enzymes. The ECM components of the decellularised nerves have been identified using H&E, Sirius red, Millers Elastin and Alcian blue staining. DAPI staining was used to identify cell nuclei.

Results

Results show the elimination of cellular components and preservation of native nerve architecture (Figure 1). The ECM components collagen, elastin and glycosaminoglycans remained intact throughout the procedure. Future work includes optimisation of the decellularisation protocol, additional scaffold characterisation and repopulation with Schwann cells.

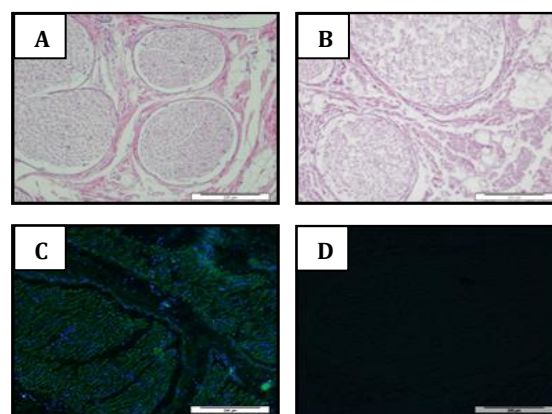


Figure 1. Decellularisation of porcine sciatic nerve. H & E of (A) fresh and (B) decellularised nerves. Nuclei staining by DAPI and fluorescence microscopy of (C) fresh and (D) decellularised sciatic nerve. Scale bar = 100µm.

Discussion and Conclusions

Porcine peripheral nerves have been successfully decellularised using a proprietary technique. The acellular model can be used as a basis for the study of perfused flow within the tissue for the introduction of primary Schwann cells. Key questions can additionally be asked such as the influence of a native 3D environment on cell migration and development.

References

1. Dunning C, McArthur SL, Haycock JW (2010) Three-Dimensional Alignment of Schwann Cells Using Hydrolysable Microfiber Scaffolds: Strategies for Peripheral Nerve Repair. *Methods in Molecular Biology*. 695:155-66.
2. Bell JHA, Haycock JW (2012) Next generation nerve guides - materials, fabrication, growth factors and cell delivery. *Tissue Engineering* 18(2):116-28.

Acknowledgments

This work is funded by the EPSRC.

Living biointerfaces to direct cell function

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^{3*} MiMe lab, Division of Biomedical Engineering, University of Glasgow, UK,

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INTRODUCTION:

Biomaterials surfaces have been functionalised with a broad variety of proteins and fragments seeking to direct cell response. However, these passive coatings cannot provide the dynamic cues that cells find in the natural ECM. We hypothesised that non-pathogenic bacteria can provide such a role, as they can colonise the surface of a broad range of materials and can be genetically modified to express desired adhesive proteins or factors to a living cell population, upon demand. This work introduces a living interface based on *Lactococcus lactis* expressing a FN fragment (FNIII₇₋₁₀) as a membrane protein.²

METHODS:

The pGFP-C2 plasmid was used to construct a vector with FNIII₇₋₁₀ downstream to GFP. FNIII₇₋₁₀ fragment was amplified and cloned into the PT1NX plasmid. Transformed bacteria were cultured on glass surfaces. The density of FNIII₇₋₁₀ was quantified using ELISA with monoclonal antibodies. C2C12 myoblasts were cultured on the living interface for up to 4 days. Cell adhesion and signalling was investigated by staining focal adhesions (vinculin) and FAK phosphorylation. Cell differentiation was quantified by staining sarcomeric myosin.

RESULTS:

We engineered food-grade *Lactococcus* to present the FNIII₇₋₁₀ fibronectin fragment as a membrane protein, containing RGD and PHSRN synergy sequences and promotes cell adhesion and differentiation (Fig. 1).

Cells adhere, spread, develop focal adhesions and promote the FAK phosphorylation on the FN-expressing *L. lactis*, in a similar way as when seeded directly on a FN coating. The myogenic differentiation triggered by *L. lactis*-

FNIII₇₋₁₀ strain was found higher compared to the non FN-expressing control strain and higher than on standard myogenic differentiation substrates (collagen I and fibronectin coatings).

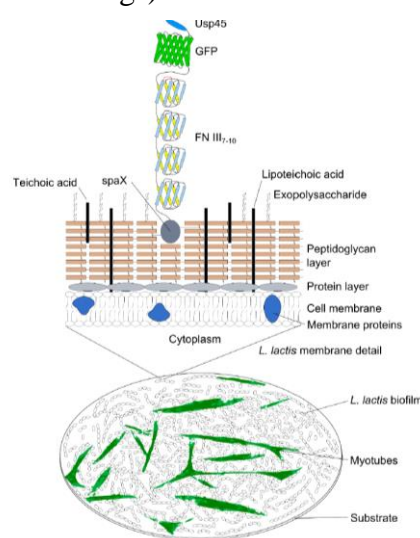


Figure 1. Schematic of the living biointerface. C2C12 myoblasts differentiated to myotubes on this system

DISCUSSION & CONCLUSIONS:

We have shown that non-pathogenic bacteria based on *L. lactis* expressing the FNIII₇₋₁₀ fragment form a dynamic biointerface between synthetic materials and cells. Overall, this living interface enhances myogenic differentiation. Further genetic modification of this living interface is being done to engineer a dynamic which can be then applied to several strategies to promote tissue repair and regeneration.

REFERENCES:

1. M. Lutolf et al (2009), Nature **462**:433.
2. A. Saadeddin et al (2013) Adv Health Mater **1213**: 2013

ACKNOWLEDGMENTS:

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The Effect of Long-term 3D Culture on Cell Morphology and Behaviour

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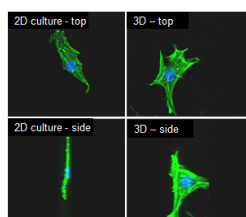
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INTRODUCTION:

Conventionally cells are grown and passaged in 2D. However, 2D growth substrates force cells to flatten and lose their characteristic morphology (Fig 1)¹. Thus, the artificial nature of 2D culture can produce physiologically irrelevant observations of cell behaviour². Using commercially available porous polystyrene scaffolds, a method has been developed to propagate cells continually in 3D, allowing for an observation of the long-term adaptation cells undergo when placed in an *in vivo*-like environment. This is a fundamental question in cell biology and understanding the adaptation process will elucidate tissue formation. The hypothesis behind this work is that changes in focal adhesion signalling networks lead to cytoskeletal reorganisation, and downstream perturbation in gene expression, directly linking a more tissue-like morphology to more physiological cell behaviour.

Fig. 1. Cells show a more realistic tissue-like morphology when grown in 3D culture, as indicated by a change in cytoskeletal staining (Phalloidin).



METHODS:

HepG2 cells were seeded onto 6 well Alvetex® Strata inserts at a density of 5×10^5 and grown for 5 days. Cells were removed, counted, re-suspended and plated onto fresh inserts every 5 days. This process was conducted in parallel with HepG2 cells maintained in 2D culture. The structural phenotype of cells passaged in 2D and 3D was analysed. Statistical significance presented as mean \pm SEM.

RESULTS:

Cells maintained in 3D show rounder morphologies than their 2D counterparts (Fig 2A); this increase in circularity is maintained when cells are re-introduced into 2D, indicating that changes are hard-wired into the cell cytoskeleton. This in turn may prime cells in preparation for 3D culture or transplantation. Such adaption was shown by

introducing 2D and 3D maintained cells into an aggregate model (Figs 2B and 2C). Cells propagated in either 2D or 3D adapted and behaved differently: 3D primed cells show markedly reduced expression of phosphorylated FAK (pFAK) (a key molecule in cell-ECM signalling and indirectly involved in control of cell shape) (Fig 2D).

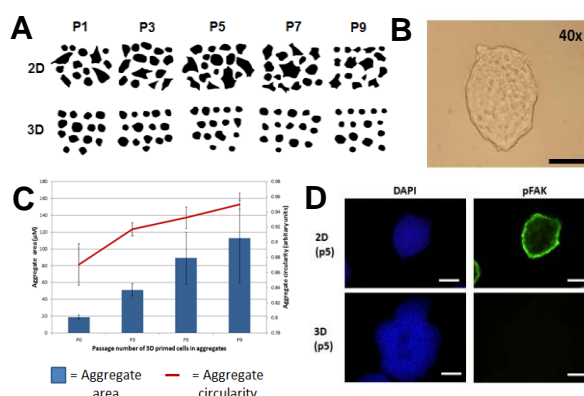


Fig. 2. Cells on Alvetex® Strata adapt to the 3D micro-environment. Cytoskeletal reorganization over the adaptation period allows cells to acquire a rounder phenotype (A). Cells become primed to 3D culture environments (B&C), and show differences in FAK signaling (D).

DISCUSSION & CONCLUSIONS:

Cells primed for 3D growth maintained morphologies more closely resembling cells in primary tissue. A reduction in pFAK in 3D appears to be due to a switch in signalling pathway brought about by the change in physical micro-environment. Differences seen in aggregates grown from 2D and 3D primed cells are possibly due to changes in cell adhesion and cell-ECM signalling, a hypothesis supported by the change in pFAK expression. Further work into this pathway will show how cells adapt to 3D culture. Such mechanisms are fundamental to understanding tissue formation.

REFERENCES:

¹ M. Schutte *et al.* (2011) *Assay Drug Dev. Techn.* **9(5)**:475-486. ² C.S. Chen *et al.* (2005) *MRS Bulletin* **30(3)**:194-20

ACKNOWLEDGMENTS:

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Dynamic Surfaces for Modulating Stem Cell Fate

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INTRODUCTION:

Enhanced control over stem cell fate would be advantageous for supply of stem cells and mature cell types. Some chemistries [1] and topographies have been harnessed to retain stem cell character or stimulate differentiation, but triggering an on demand ‘switch’ from retention of stemness to maturation would be valuable to promote expansion prior to differentiation.

METHODS:

Human mesenchymal stem cells (hMSCs) were cultured on peptide modified glass coverslips, presenting adhesive –RGD sequences adjacent to an –FMOC or –PEG blocking group attached to an elastase-cleavable linker. Surfaces could be enzymatically ‘switched’ to present the concealed –RGD. Glass, –RGE, –RGD, and –PEG presenting surfaces were controls. Focal adhesions (FAs) were quantified to evaluate cell adhesion, and cells were examined for stem and differentiation markers (Stro-1, adipo- and osteo markers). For metabolomics, solvent-extracted metabolites were analysed using an Orbitrap Exactive mass spectrometer. Ingenuity Pathways Analysis was used to evaluate the functional implications of the differentially abundant metabolites.

RESULTS:

The stem cell marker Stro1+ was retained more effectively on unswitched surfaces relative to switched surfaces after 2 and 4 weeks of culture. The cells were still multipotent and could be induced to differentiate into other lineages on the unswitched surfaces in the presence of induction medium. On switched surfaces, where greater cytoskeletal tension was promoted and –RGD was exposed, FAs were modulated, and osteogenesis was induced. The

metabolic response was distinct, with MSCs on switched surfaces showing a more metabolically active, differentiating phenotype. Unsaturated carbon-carbon double bonds are associated with stem cell plasticity [2]. Cells were depleted of unsaturated lipids on switched substrates, and these were enriched on unswitched surfaces (Fig. 1), consistent with stem cell retention.

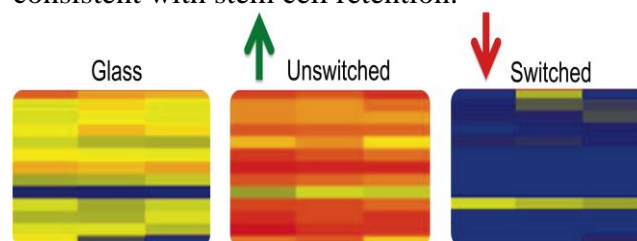


Fig. 1: Abundance levels of various unsaturated lipid metabolites, generally enriched (orange) on unswitched (-RGD concealed) surfaces and decreased (blue) on switched (-RGD exposed) substrates, relative to controls.

DISCUSSION & CONCLUSIONS:

The stem cell phenotype was retained more effectively on unswitched surfaces relative to switched surfaces, and cells retained their multipotency after culture on unswitched substrates. The switched surfaces could modulate FAs and promoted osteogenesis. The system offers potential for the retention of stem cell phenotype and controlled differentiation.

REFERENCES:

1. Curran et al (2005), *Biomaterials*. **25**:7057-67.
2. Yanes et al (2010), *Nat. Chem. Biol.* **6**: 411-7.

ACKNOWLEDGMENTS:

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Characterisation of distinct regional and immuno-selected cell populations derived from human foetal femurs and their capacity for bone defect repair and regeneration

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Introduction

Skeletal stem cells (SSCs) isolated from adult human bone marrow (HBM) and utilised for bone tissue engineering strategies remain heterogeneous, exhibiting variable proliferation and differentiation capacity¹. Here the authors investigate human foetal femurs as an alternative stem cell source². Initial studies investigated regional derivation of cell populations and assessment of their osteochondral differentiation capacity. SSCs were subsequently identified and isolated according to Stro-1 expression. Enriched populations were assessed for osteochondral differentiation and bone defect regeneration.

Materials and Methods

Femurs were dissected yielding epiphyseal and diaphyseal populations. Stro-1+ cells were immuno-selected from whole femurs by MACS. Proliferation and differentiation capacities within isolated populations were assessed *in vitro* for alkaline phosphatase activity and colony formation. Osteochondral differentiation was assessed through molecular and histological analysis. Tissue formation and regeneration were assessed *in vivo* via subcutaneous diffusion chamber implantation within immunodeficient mice, and *ex vivo* via cell pellet implantation within chick femur drill defects.

Results

Distinct epiphyseal and diaphyseal populations exhibited robust chondrogenic and osteogenic differentiation potential respectively, according to ALP+ colony formation, osteochondral gene expression, *in vivo* tissue formation, and *ex vivo* bone defect regeneration. Stro-1+ cells isolated from the diaphyseal region of foetal femurs (dependent on developmental stage) exhibited prolonged phenotype stability (Stro-1 from P1-P6), self-renewal, osteo, chondro and adipogenic differentiation, and tissue formation/defect regeneration capacity.

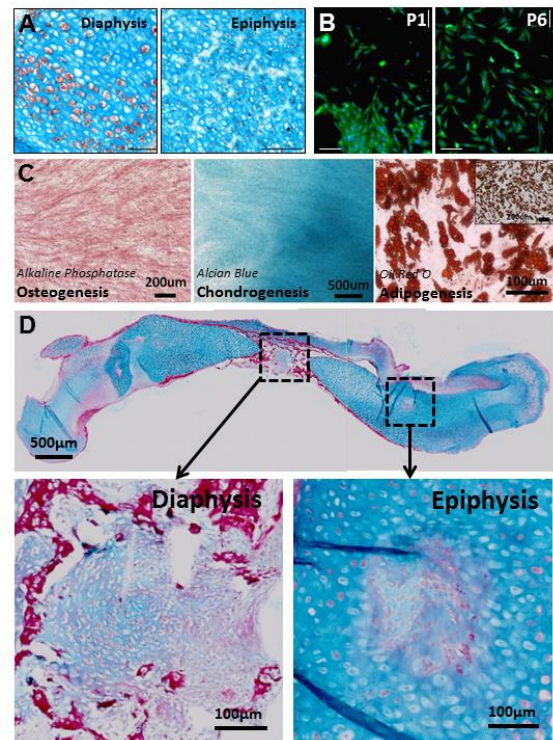


Figure 1: Diaphyseal Stro-1+ cells (A) exhibit prolonged Stro-1 expression from passage 1 to 6 (B), multi-lineage differentiation (C) and defect regeneration (D).

Discussion and Conclusions

Epiphyseal and diaphyseal cells offer robust chondrogenic and osteogenic populations, whilst Stro-1+ cells offer a SSC source for interrogation of bone biology prior to clinical translation.

References ¹ K. Schallmoser, Haematologica 95 (6) 867-874 2010. ² S. H. Mirmalek-Sani, Stem Cells 24 (4) 1042-1053 2006.

Acknowledgments

We thank surgeons at Southampton General Hospital for provision of foetal tissue. This work was funded by the BBSRC (BB/GO10579/1).

Disclosures

No conflicts of interest exist.

Mesenchymal Stromal Cells Organise Endothelial Networks through PDGF, ILK and FGF Signalling

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INTRODUCTION: In addition to their capacity for skeletogenic differentiation, there are suggestions that mesenchymal stem/stromal cells (MSCs) have additional roles in organising tissue vasculature through interactions with endothelial cells (ECs). However, suitable experimental models to test these unique MSC activities are lacking and the mechanisms are unclear. Here, we have developed a novel 3D in vitro co-culture spheroid model of MSCs and ECs to track endothelial restructuring and identify the signalling processes involved.

METHODS:

3D co-culture spheroids totalling 30,000 cells were produced using a 50:50 mixture of human MSCs and ECs. The cells were labelled using CellTracker™ green and red respectively before being placed in non-adherent U-bottomed 96 well plates in appropriate medium containing 0.25% (w/v) methyl cellulose. The 3D MSC:EC spheroids were cultured in the presence and absence of a range of specific inhibitors of different signalling pathways, ultimately focusing on platelet-derived growth factor receptor (PDGFR), integrin-linked kinase (ILK) and fibroblast growth factor (FGF). Spheroids were imaged by multiphoton and fluorescence microscopy following sectioning.

RESULTS:

Between day 1 and 2 of culture, dramatic self-organisation was observed in MSC:EC spheroids. ECs formed inter-connected vascular like lattices surrounded by MSCs, which extended from peripheral EC assemblies to internal networks throughout the spheroid (Fig. 1A, left column) compared to the random distribution of MSC:MSC spheroids (Fig1A, right column). All inhibitors of PDGFR, ILK and FGF disrupted spheroid self-organisation by altering the peripheral distribution of ECs and causing them to form enlarged internal cell aggregates without the connected lattices observed in untreated controls (Fig.1A). This was particularly

pronounced for FGF inhibition and confirmed by image analysis quantification. Exposure to PDGFR and FGF inhibitors significantly increased the spheroid volume (Fig-1B) and image analysis revealed that ILK inhibition significantly increased the percentage of MSCs at the spheroid periphery (Fig. 1C).

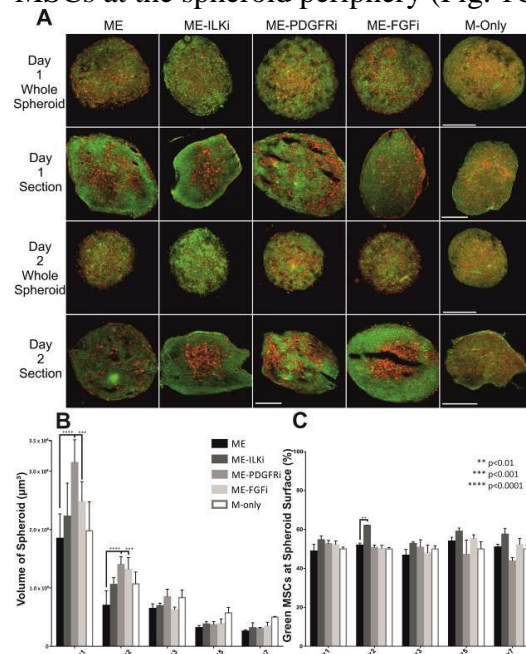


Fig 1: MSC:EC (ME) spheroids were cultured with and without ILK, PDGFR and FGF inhibitors A – Whole spheroid images and sections show organisation at days 1 and 2 (MSC:EC). Scale bars = 200µm. B – Volume of spheroids over 7 days. C – Percentage of MSCs (green) at the spheroid periphery over 7 days.

DISCUSSION & CONCLUSIONS:

This study has showed that MSCs and ECs have an intrinsic capacity to self-organise when co-cultured under defined 3D conditions to form elaborate vascular-like networks in a mechanism that is dependent on PDGF, ILK and FGF-mediated signalling. Our novel 3D co-culture model represents a simplified system to decipher the mechanisms guiding MSC-dependent remodelling of host vasculature, which may be exploited to augment MSC-based tissue repair.

ACKNOWLEDGEMENTS: The authors would like to thank the EPSRC for funding.

Modulation of Human Skeletal Stem Cell Fate and Function as a Consequence of Nanotopography and Hypoxia

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INTRODUCTION:

Degenerative skeletal diseases pose a significant socioeconomic burden in aging populations. Skeletal stem cell (SSC) based therapies may enhance current treatment strategies. Essential to this approach is SSC manipulation *in vitro*. Conventional protocols employ chemicals to direct SSC fate. This increases patient risk and reduces the chance of successful clinical translation. Nanotopography and hypoxia are critical components influencing the SSC niche *in vivo* and have been shown, individually, to influence cell behaviour *in vitro*. We have examined the influence of nanotopography and hypoxia to modulate SSC fate and function as alternatives to chemical cues.

METHODS:

Fetal and adult SSCs were cultured on square (SQ; Fig.1B) and near square (NSQ50; Fig.1C) arrangements of nanopits, 120nm diameter, under 2% and 20% oxygen. Osteogenic and adipogenic differentiation were analysed by qPCR, immunofluorescence and Oil red O staining. Label-free coherent Raman microscopy was used for lipid detection.

RESULTS:

Cell tracker green staining indicated excellent viability of human fetal SSCs on nanotopographical substrates (Fig.1D)

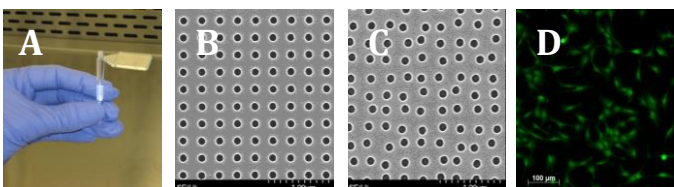


Fig.1A Nanotopographical substrate produced by electron beam lithography and injection molding. **B&C** Electron micrographs of SQ and NSQ nanopatterns. **D** Viable human fetal SSCs on nanotopography.

In contrast to adult SSCs^{1,2} and ESCs³, the nanotopographies investigated had no effect on human fetal SSC fate. However hypoxic conditions influenced both fetal and adult SSCs with a decrease in expression of osteogenic genes ALP and OCN and an increase in VEGF expression. On tissue culture plastic Coherent Raman microscopy provided highly sensitive lipid detection compared to conventional Oil Red O Histochemistry. This allowed earlier detection of adipogenic differentiation of fetal and adult SSCs (Fig.2).

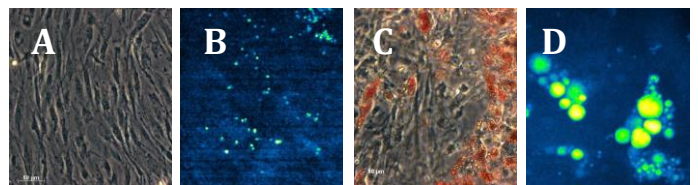


Fig.2 Lipids (yellow) detected by Coherent Raman imaging (2B) but not Oil Red O staining (2A) by day 3 of differentiation. 2C&D: Day 14.

DISCUSSION & CONCLUSIONS:

The mechanisms underlying the clear differences between adult and fetal SSC response to defined nanotopographies are currently under investigation. Key to tissue regeneration strategies is vascularization of the new tissue. Therefore the increase in VEGF expression under hypoxia is of particular interest and is also currently under examination.

REFERENCES:

1. Dalby M. et al. (2007) Nature Materials. 6:997-1003;
2. McMurray R. et al. (2011) Nature Materials 10:637-644;
3. Kingham E. et al. (2013) Small 9(2):2140-2151.

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The “Cryptic” Extracellular Matrix and Its Impact on Stem Cell Differentiation

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INTRODUCTION:

Important signaling pathways governing cell migration and differentiation are initiated by the extracellular matrix (ECM), which constitutes the initial barrier, the actual tissue boundary for every cell. Migration and differentiation, such as the epithelial-to-mesenchymal transition (EMT), are the main factors in development and interactions between ECM proteins and cells are key players in initiating cellular changes. The proteolytic remodeling of ECM proteins results in the release of ECM fragments hidden within the tertiary structure – so called “cryptic” sites. In recent years, more and more cryptic ECM fragments could be identified and their importance in understanding development has been highlighted (1).

METHODS:

Expression patterns of matrix metalloproteinase-2 (MMP2) has been investigated in human and mouse embryonic stem cells (ESCs) by qPCR and protein activity assays. Laminin-111 has been processed with MMP2 and fragments were studied by mass spectrometry and peptide fingerprinting. Impact of the fragment on the differentiation of human and mouse ESCs has been assessed by qPCR and protein expression.

RESULTS:

We could identify a biologically active laminin-111 fragment that was exposed through cleavage by MMP2 from the N-terminal region of the β 1-chain. This fragment is able to interact with α 3 β 1-integrin/CD147 and to decrease expression of MMP2 and increase expression of E-cadherin – two key players in the EMT. We could show that a cryptic laminin fragment is directly involved in EMT signaling (2).

DISCUSSION & CONCLUSIONS:

Here we report a previously unidentified laminin fragment that is released by MMP2 processing. This cryptic fragment induces changes in the EMT of mouse and human ESCs and could be used in various biomedical applications, where the modulation of the EMT is desirable.

REFERENCES:

1. Schenk S, Quaranta V (2003) Tales from the crypt[ic] sites of the extracellular matrix. *Trends Cell Biol* 13(7):366-375.
2. Horejs C-M, Serio A, Purvis A, Gormley AJ, Bertazzo S, Poliniewicz A, Wang A, DiMaggio P, Hohenester E, Stevens MM (2014) A biologically active laminin-111 fragment that modulates the epithelial-to-mesenchymal transition in embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.*, doi:10.1073/pnas.1403139111

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Engineering the corneal stroma: The effect of a three-dimensional environment and growth factor supplementation

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INTRODUCTION: A major challenge of producing a tissue-engineered cornea is the recapitulation of the complex environment of the stroma. The resident cells of the stroma are keratocytes; a quiescent, dendritic cell, responsible for maintaining the extracellular matrix (ECM). Upon injury, or during *in vitro* culture in foetal bovine serum (FBS), keratocytes differentiate into a fibroblastic repair phenotype¹. Differentiating these fibroblasts back to keratocytes may be crucial in stromal tissue-engineering. Methods that have been suggested are three-dimensional environments and serum-free medium^{2,3}.

METHODS: Primary human corneal stromal cells (CSC) were cultured to achieve a fibroblastic cell type reminiscent of an MSC. CSC were seeded in poly(lactic-co-glycolic acid) (PLGA) microfibre scaffolds. Constructs were maintained under 4 media conditions: 20% FBS; 2% FBS; insulin-transferrin-selenium supplementation (ITS); and bFGF and TGF- β 3 supplementation (GF). Proliferation, cell phenotype, gene expression and matrix production were assessed.

RESULTS: Scaffolds (fibre diameter: 1-3 μ m) supported cell adhesion and proliferation in all media types (fig. 1). Gene expression and immunocytochemistry show that scaffolds in ITS and GF media promote the keratocyte phenotype with increased expression of keratocyte markers CD34 and ALDH3A1.

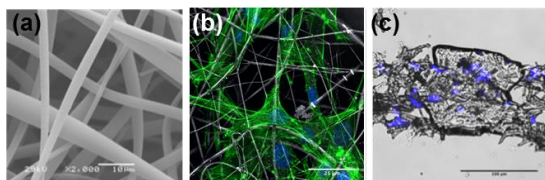


Figure 1: (a) Scanning electron micrographs of microfibrils. (b) F-actin staining shows dendritic morphology of cells. (c) Cross sections of constructs stained with DAPI show cellular infiltration.

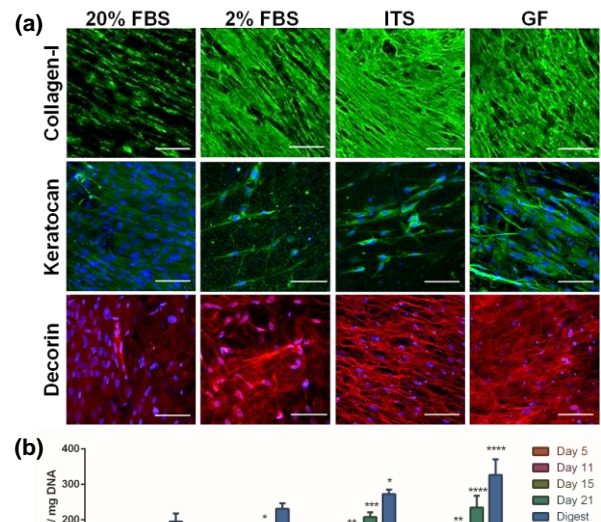


Figure 2: (a) Immunocytochemistry of collagen-I, keratocan and decorin on scaffolds. (b) Cumulative production of glycosaminoglycans (GAGs) released into medium and when scaffold digested at day 21.

Collagen-I deposition was seen predominantly in ITS/GF cultures (fig. 2a). Expression of proteoglycans such as keratocan and decorin were greatest in GF. GAG production was highest in GF (fig. 2b).

DISCUSSION & CONCLUSIONS: The PLGA microfibre scaffolds allowed infiltration of CSCs and promoted a keratocyte phenotype. This effect was augmented with the use of serum-free medium and growth factor supplementation, which led to improved ECM production and deposition.

REFERENCES:

1. Poole et al. (1993), *J Cell Sci*, **106**: 685–91.
2. Kawakita et al. (2006), *IOVS*, **47**:1918-27.
3. Wu et al. (2013), *Tissue Eng*, **19**:2063-75.

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Hypermethylation in OA - Transactivation of the *COL9A1* proximal promoter region by SOX9 in chondrocytes is regulated by DNA methylation

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INTRODUCTION:

Methylation of genomic DNA represents a significant mechanism for regulating tissue-specific gene expression. SOX9, a transcription factor pivotal in chondrogenic differentiation¹, activates *COL9A1* gene expression². However, the role of CpG methylation in *COL9A1* transactivation by SOX9 remains unknown.

METHODS:

The ChIP-IT Express Enzymatic Kit (Active-Motif) was used to perform ChIP assays using C28/I2 chondrocytic cells co-transfected with non-methylated or methylated pCpGfree-Luc-COL9A1 vector (-846/+128bp) as well as the expression vector encoding SOX9 using Fugene HD (Promega). 48 hours after transfection, pre-cleared chromatin was stored as assay input or incubated at 4°C overnight with 5 µg of rabbit anti-SOX9 antibody (Abcam) or normal rabbit IgG (Cell Signalling). After reverse cross-linking and purification, the final DNA preparations were subjected to quantitative PCR analysis using 5µl of the eluted DNA. For real time PCR analysis, the CT of each sample was normalized to the CT of the input sample. Statistical Analysis: Results presented as mean ± S.E. of four independent experiments. Statistical significance was evaluated using Mann-Whitney U test, with $p < 0.05$ considered significant.

RESULTS:

ChIP assays revealed that methylation treatment significantly reduced SOX9 binding to the *COL9A1* promoter (Fig. 1), specifically two binding sites close to the transcription start site (BS4 and BS5). These results show decreased SOX9-driven *COL9A1* transactivation after methylation treatment of the wild type (WT) *COL9A1* reporter construct.

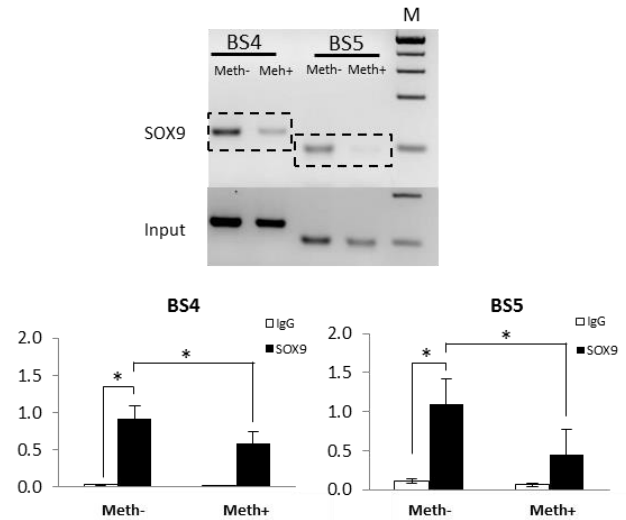


Fig. 1. ChIP assays were performed using cell lysates of C28/I2 cells transfected with non-methylated (Meth-) or methylated (Meth+) WT -846/+128-bp *COL9A1* promoter constructs and the expression vector encoding SOX9. * $p < 0.05$

DISCUSSION & CONCLUSIONS:

We show for the first time that in human chondrocytes CpG methylation of the *COL9A1* proximal promoter specifically impairs SOX9-driven promoter activation by altering SOX9 binding to DNA. Furthermore, transactivation depended on the DNA methylation status of two SOX/Sry binding sites (BS4 and BS5). This is the first demonstration that hypermethylation is associated with down-regulation of *COL9A1* expression in OA indicating the pivotal role of epigenetics in decreased anabolism in OA.

REFERENCES:

1. Kim KI, Park YS, Im GI. *J Bone Miner Res* 2013;28(5):1050-60.
2. Zhang P, Jimenez SA, Stokes DG. *J Biol Chem* 2003 3;278(1):117-23.

ACKNOWLEDGMENTS:

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Photochemical Functionalisation and Direct-Write Patterning of Diamond-Like-Carbon for Neural Interfaces

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INTRODUCTION:

Electrodes for direct interface with the brain have the potential to greatly improve prostheses to aid victims of spinal injury or similar disablement. However, the development of such electrodes relies on careful engineering of the biological-electronic interface with control of scarring, cell phenotype and tissue organisation. Diamond-like-carbon (DLC) may be an ideal material for coating electrodes as it has flexible electrical properties, it is extremely stable in vivo and is already used as a coating for orthopaedic implants¹. Chemical modification is essential, however, to control and promote adhesion, differentiation and organisation of neurons and glia.

METHODS:

DLC was coated on silicon by chemical vapour deposition. DLC was rendered either non-adhesive by surface initiated atom transfer radical polymerisation of PEG brushes or cell-adhesive by photochemical polymerisation of charged monomers such as acrylic acid or amine functional acrylates. Surfaces were also patterned by direct-write laser induced photopolymerisation using a microchip Nd-YAG nanosecond pulsed laser in order to provide spatial control of cell adhesion. Rat neural stem cells were seeded to the surfaces and allowed to spontaneously differentiate.

RESULTS:

Modified DLC surfaces were characterised by XPS and contact angle. Hydrogenated DLC and PEG-functionalised DLC were found to be non-adhesive to cells. By modification with surface initiated charged polymers, hydrophilicity and cell adhesion were promoted. Neural progenitors adhered to the modified surfaces and a multipotent phenotype was maintained until withdrawal of growth factors. Spontaneous differentiation occurred on the modified surfaces with both neuronal

and glial differentiation observed. Patterned tracks were prepared by direct-write polymerisation of charged monomers and were characterised by AFM.

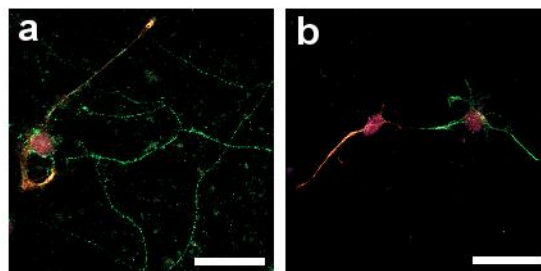


Fig. 1. Immunofluorescence micrographs of neural progenitors after spontaneous differentiation on polyacrylic acid coated surfaces. Cells were stained for nestin (red), β III-tubulin (green, c) and GFAP (green, d). Scale = 80 μ m.

DISCUSSION & CONCLUSIONS:

The surface chemistry of DLC was modified and patterned by facile photochemical methods. Such approaches allow for the tuning of biological properties and the controlled design of the electrode-brain interface. Further studies on the differentiation of neural stem cells and the development of controlled patterning protocols will be carried out.

REFERENCES:

1. Regan, EM et al. *Biomaterials* (2010), 31, 2, 207-215.

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Influencing the Skeletal Stem Cell Niche by Targeted Liposomal Wnt Delivery

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INTRODUCTION: Bone fracture treatment costs the UK billions of pounds each year. New regenerative medicine therapies for bone tissue are urgently required. Skeletal stem cells (SSCs) of the bone marrow niche are an attractive target for such new technologies, therefore we hypothesise that delivery of growth factors specifically to SSCs may enhance bone regeneration. The Wnt signalling pathway has been shown to regulate SSCs¹, hence we aim to deliver Wnt proteins, entrapped in liposomes, targeted to SSC marker STRO-1². Our previous unpublished data showed that short-term induction of Wnt signalling increased the population of stem cells and promoted generation of early and late osteoprogenitors. However, long-term Wnt induction had the opposite effect, indicating that Wnt signalling has strikingly different effects on bone cells depending on their osteogenic commitment¹. We think Wnt entrapment in liposomes is a preferable delivery method (Fig. 1) for such a hydrophobic growth factor, and will ensure the specificity and therefore safety and efficacy of our approach.

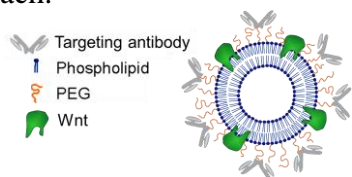


Fig. 1 Proposed therapeutic liposome design.

METHODS: We characterised the fabricated liposomes' stability and size *via* dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). We analysed the lipid content of our preparations by gas chromatography (GC). We also measured by FACS the uptake of these nanoparticles by BMMNCs and the biological activity of liposome-incorporated Wnt3a protein on a reporter cell line. All statistical analysis was conducted using GraphPad Prism software with significance set at $p < 0.05$.

RESULTS: Nanoparticle preparations were 100 ± 20 nm in size and $>80\%$ of BMMNCs were able to take up these particles within 1h of incubation (Fig. 2).

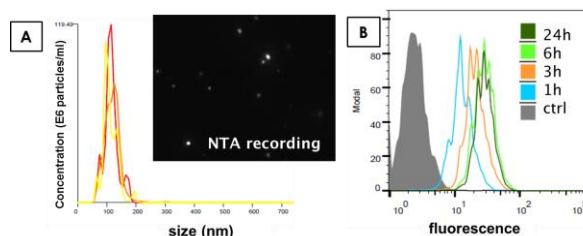


Fig. 2 Liposomes were 100 ± 20 nm in size as measured by NTA (A) and were taken up rapidly by BMMNCs as assessed by FACS (B).

The biological activity of Wnt3a incorporated in liposomes was dependent on their lipid composition. Liposome preparations consisting only of the DMPC lipid were active and induced a response similar to neat Wnt3a protein. However PEGylation of the DMPC lipid preparations abolished the activity of Wnt protein. This was not the case when the DOPC lipid was used, as both unPEGylated and PEGylated preparations were active.

DISCUSSION & CONCLUSIONS: Based on our previous unpublished data on Wnt effects on SSCs and that liposomal Wnt delivery is the method of choice for highly hydrophobic proteins³, selective targeting of Wnt proteins in liposomes to specific cell populations at a specific differentiation stage might be a safe and efficacious therapeutic approach for promoting bone regeneration. Future experiments must include better characterisation of the chemistry of Wnt protein incorporation into the liposomal bilayer with regards to suitability for the novel targeted delivery approach within the bone marrow stem cell niche.

REFERENCES:

1. J de Boer et al (2004), *Bone* **34**(5): 818-826.
2. PJ Simmons et al (1991), *Blood* **78**(1): 55-62.
3. GR Dhamdhare et al. (2014), *PLoS One* **9**(1): e83650.

Clay gels localise and enhance BMP2 induction of osteogenesis

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INTRODUCTION:

Bone Morphogenic Protein (BMP) is a licensed therapy to enhance bone repair, however delivered BMP rapidly diffuses and degrades requiring supra-physiological doses for efficacy. The synthetic clay Laponite, is non-toxic with a 25 year history of use in cosmetics. We have shown its potential to form hydrogels able to localise biological molecules to induce regenerative responses^{1, 2}. Here we demonstrate *in vitro* and *in vivo* the utility of this approach to localise BMP2 to enhance bone graft osteo-induction.

METHODS:

Clay gels were formed using hydrous suspensions of the synthetic clay Laponite (Rockwood Ltd.). The effect of clay gels on BMP-2 localisation and activity was explored *in vitro* using C2C12 cells and staining for alkaline phosphatase activity (APA). Clay gels were spotted on tissue culture plastic (TCP) and BMP-2 was added to the media before or at the time of cell seeding. After 72 hours culture, staining and image analysis for APA was performed. Effect of Laponite, BMP-2 and serum concentration, seeding density and BMP-2 incubation time was assessed. C2C12 BMP-2 response on clay gel coated decellularised bone graft (DBG) was also assessed. *In vivo*, a Laponite-BMP-2 mix perfused into DBG was subcutaneously implanted in mice. New bone formation versus controls was assessed at 28 days using micro-computed tomography (μ CT).

RESULTS:

Induction of APA was significantly ($p < 0.001$) enhanced on clay gel surfaces over tissue culture plastic (fig. 1a) in a BMP-2 dose-dependent manner. Enhanced response was localised to clay gels, independent of local cell density and attenuated by increased serum concentration. Enhanced BMP2 induction of

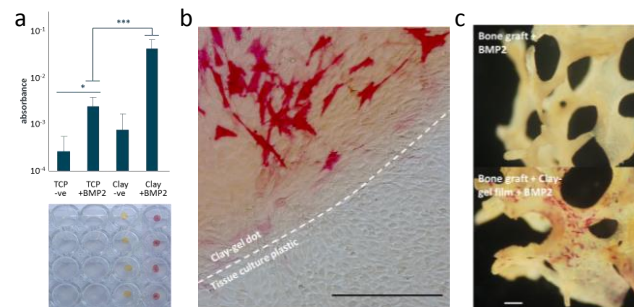


Figure 1. Laponite gels spotted upon tissue culture plastic localised and enhanced APA in C2C12 cells by BMP2 (a-b). The same approach enhanced BMP2 response in cells seeded upon clay-coated clinical grade bone graft (c). Scale=250 μ m.

APA was also observed in C2C12 cells seeded onto trabecular bone graft pre-coated with a Laponite gel (fig. 1b). Pre- and post-implantation μ CT analysis of bone graft + BMP2 with or without clay gels demonstrated a significant increase in bone volume ($p < 0.03$) bone volume per tissue volume, ($p < 0.02$), and trabecular number ($p < 0.03$) in graft material treated with BMP2 + Laponite gels. No significant increase in bone was observed on graft material treated with BMP-2 or Laponite alone.

DISCUSSION & CONCLUSIONS:

These studies provide *in vitro* and *in vivo* evidence for the utility of Laponite gels to enhance induction of osteogenic responses via the localisation of BMP2.

REFERENCES:

1. J.I. Dawson et al (2011) Advanced Materials **23**: 3304-8.
2. J.I. Dawson & R.O.C. Oreffo (2013) Advanced Materials **25**: 4069-86.

ACKNOWLEDGMENTS:

We gratefully acknowledge EU (FP7) Biodesign and Rosetrees Trust for funding this work.

Nano-Kicking stem cells into making Bone

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INTRODUCTION:

Mesenchymal stem cells (MSCs) have large regenerative potential to replace damaged cells from several tissues along the mesodermal lineage. [1] Controlled differentiation of these cells can be brought about using various physical environmental conditions *in vitro*[2] Here, we present data demonstrating our ability to control these cells at a nano-level, and induce osteoblastogenesis in MSCs using high frequency (1000 Hz, 3000 Hz & 5000 Hz) piezo driven nano-displacements coupled with a nano-topographical (NSq-50) surface.

METHODS:

MSC were exposed to high frequency nanoscale displacements with and without the presence of a nano-topographical (NSq-50) surface. Using negative and positive controls (osteogenic media) the cells were assessed for the onset of osteogenesis following the flow of biological information from a genomic/transcriptomic level, to the proteomic level and finally at a $\text{Ca}_3(\text{PO}_4)_2$ mineralisation level. To determine this qRT-PCR (ANOVA), immunostaining, in cell western, Raman spectroscopy and histological stains (Alizarin Red & Von Kossa) were performed at varying time points.

DISCUSSION & CONCLUSIONS:

High frequency piezo stimulation is a novel way to induce MSC differentiation into osteoblastic cells, giving rise to a high level of efficacy through focal adhesion manipulation at the nano-scale.[2] Having interrogated an optimum condition in 2D it is envisaged that presently ongoing research in 3D gels could be used to develop a vibrational bioreactor. This may in the future have real world practical application to provide a ready source of autologous osteoblastic cells providing a

RESULTS:

potential therapeutic effect for musculoskeletal conditions.

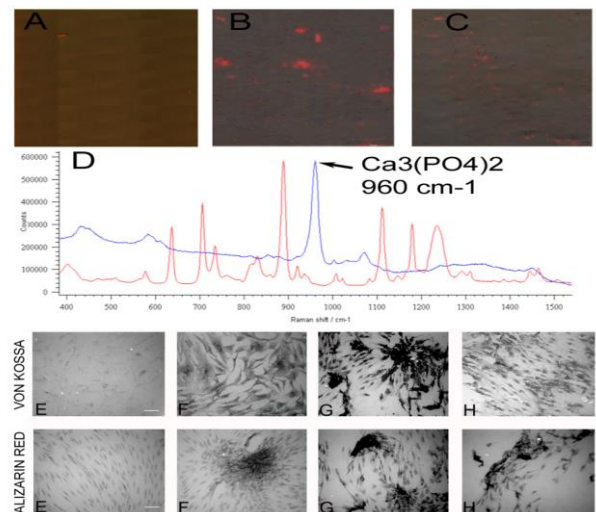


Fig. 1 : (A-C) 25 mm² Raman scans at 960 cm⁻¹, this wavenumber as shown from image (D) is the main peak observed in the fingerprint region of bovine cortical bone. (A) blank surface (B) 1000 Hz on a flat surface (C) 5000 Hz on NSq-50, these are assessed after 27 days of continuous piezo stimulation. (E-H) Von Kossa & Alizarin stains performed after 34 days of continuous piezo stimulation. (E) blank surface, (F) NSq-50 surface only, (G) 1000 Hz piezo only, (H) 5000 Hz and NSq-50 surface. Mag x 10, scale bar = 100 um.

A vibrational frequency of 1000 Hz, by all three techniques shows the distinctive deposition of Ca_3PO_4 nodules, which confirms osteoblast differentiation.

REFERENCES:

1. Adrian K. K. TEO, L. V., *Biochem. J.* (2010) 428, **2010**, 428, 11–23.
2. Nikukar, H.; Reid, S.; Tsimbouri, P. M.; Riehle, M. O.; Curtis, A. S.; Dalby, M. J., *ACS nano* **2013**, 7 (3), 2758-67. DOI 10.1021/nn400202j.

ACKNOWLEDGMENTS:

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Adapting aligned, stabilised 3D tissues for large-scale neurobiological research

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INTRODUCTION:

Recreating the 3D environment of the CNS using hydrogel matrices allows neurons and glial cells in vitro to behave similarly to their counterparts in vivo, providing a relevant tool for neurobiological studies¹. The overall aim is to develop robust 3D CNS tissue models engineered by a process of glial cell self-alignment and subsequently stabilised. Furthermore, these models have been developed for multi-well plate format at a scale suitable for high throughput screening. CNS tissue equivalents can be used to assess numerous aspects of the CNS in a reproducible, controllable and consistent manner.

METHODS:

Characterisation studies assessed alignment and stabilisation of neurons and glia in collagen gels within a 96-well plate test rig prototype. Their potential for use in neurobiological studies involved identifying neurite growth, neuron-glia interactions and myelination following defined periods in culture. Detection and quantification analysis was conducted via immunohistochemistry and confocal microscopy.

RESULTS:

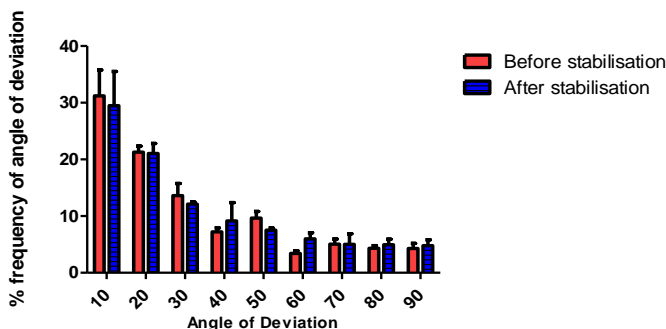


Fig 1 Alignment of neural cells persists following stabilisation of hydrogels

Hydrogels constructed within a 96-well plate rig displayed comparable cellular alignment to traditional methods using larger moulds², in mid and side regions, before and after stabilisation of constructs.

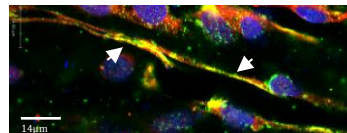


Fig 2 Confocal micrograph showing cellular alignment and neuron-glia interaction in aligned 96-well plate rig hydrogel after 14 days. Arrowheads indicate immunoreactivity for myelin basic protein adjacent to neuronal structures (Red- β -tubulin, green-MBP, blue-Hoechst).

Neurite growth was detected and measurable in the aligned tissue equivalents. Markers for myelination were identified in close proximity to neurites.

DISCUSSION & CONCLUSIONS:

Results suggest that a highly organised, stable hydrogel can be created within the dimensions of a 96-well plate. The aligned nature of the cells and extracellular matrix in this anisotropic system facilitates quantitative analysis of CNS cellular features such as neurite length and the process of myelination. This simple, consistent and physiologically relevant model system, which uses a multi-well plate format can potentially be used at a scale suitable for commercial R&D.

REFERENCES:

1. E. East et al (2012) *Tiss Eng* **16**: 3173-8.
2. M. Georgiou et al (2013) *Biomaterials* **34**: 7335-43.

Real time spatial monitoring system for 3D culture models

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INTRODUCTION: Current methods available for determining cell behaviour within 3D constructs use end point assays. Such assays destroy samples during the processing stages bringing many problems and limitations. One solution is to develop low cost techniques for non-invasive measurement of samples, providing a real time (RT) read out of 3D tissue constructs and optical approaches are ideal candidates. The Lein CTS2 is an optical measuring device capable of detecting changes in refractive index to denote surface positions in the z plane of delicate, hard to handle hydrogel constructs. Such thickness readings provide quantitative data relating to spatial changes in individual construct layers or the full construct thickness in RT. The aim of the presented work involved the characterisation and evaluation of this approach to test its potential use in compressed collagen models [1].

METHODS: Compressed collagen gels with starting collagen volumes 0.5, 1, 1.5 and 2ml were made and used to compare the CTS2 optical thickness readings with the thickness readings observed by traditional histology methods. Serially compressed 0.5ml triple layers and single layer 0.5ml gels were assembled to measure cell free re-swelling after 24h. 1ml collagen gels were compressed with hydroxyapatite particles (HA) increasing in concentration from 0.3-1.2mg total to assess sample thickness and HA concentration. Finally a complex multi-layer construct was analysed. The acellular tri-layer was assembled as a simplified bone fracture model using HA as the test material inserted into the middle collagen layer.

RESULTS: Optical RT thickness measurements were compared with those using traditional histology on the same collagen constructs. In all cases unfixed optical thicknesses were greater indicating

dehydration shrinkage. 24h Re-swelling tests indicated increased sample thickness. Addition of HA particles to constructs produced a clear increase in thickness by histology. However optical measurements did not correspond to the histology indicating a limitation to this approach. Optical measurements of the tri-layer model showed good predictability of construct thickness (fig 1) and a stable case for how to measure remodelling.

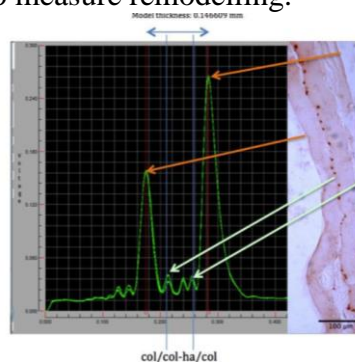


Fig.1 CTS2 optical trace for tri-layer bone fracture callus model alongside histology image (middle layer with HA particle presence stained with Alizarin Red)

DISCUSSION & CONCLUSIONS: This study has tested the potential of RT optical monitoring using the CTS2 to assess 3D native collagen tissue models. The addition of HA particles indicated a loss in signal due to light scatter leading to loss of thickness accuracy compared to histology findings. However the CTS2 is capable of measuring sample thickness changes, in this case re-swelling; further to this we can provide a non-invasive quantitative method used to analyse and map out multi-layered 3D tissue models.

REFERENCES:

1. Alekseeva, T. et al (2011), *ICCE* **30**: 5-6.

ACKNOWLEDGMENTS: Research funded by BioDesign. We would like to thank Lein applied diagnostics for their advice and expertise.

Development of a novel biorheometer for *in vitro* real time monitoring of matrix remodelling

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INTRODUCTION:

Cells perform their physiological function within a 3D context integrating instruction both from different cells and the extracellular matrix. Currently the majority of systems employed to study cell/matrix interactions are limited in their accuracy and ability to study events in real time. We sought to develop a rheological system capable of detecting biologically induced differences in matrix re-organisation of the most abundant extracellular matrix protein in humans, collagen, between wild type (WT) fibroblasts and fibroblasts lacking the GTPase Rac1 (KO), a protein known to influence cell migration.

METHODS:

Two TA Instruments ARES-G2 commercial rheometers were modified to include temperature regulation using a peltier plate (370C) and supplied with 5% CO₂/air. Furthermore a stainless steel geometry with regular holes of 2mm diameter was incorporated for media perfusion. The modified geometry showed consistency with unmodified geometries tested with standard viscosity calibration oils (not shown). Rac1 WT and KO phenotype was assessed by Rac1 RT-PCR and functionally with time lapse microscopy. The viability of cell types in 3D (type I collagen lattice) systems was assessed by viability staining (Draq7) and analysed by confocal microscopy. Rac1 WT and KO cells were maintained in DMEM/10% FBS, trypsinised and embedded at 100,000 cells/gel prior to temperature controlled gelation up to 370C. WT and KO cells were run on parallel running rheometers with incubator based cellular controls.

RESULTS:

Modifications of a commercial rheometer are shown (Fig 1a). Rac1 KO cells were confirmed to lack Rac1 expression by RT-PCR and found to show impaired migration and contraction of collagen gels (Fig 1b/c, 2a) Differences in observable matrix remodelling rates between Rac1 WT and KO cells was first evident after 3 days in culture using traditional assays of gel contraction

(Fig 2a, b). Changes in G prime were observable from 3 hours in our customised rheometer (Fig 2d) with both cell populations lacking positivity of the DNA binding dye Draq7 (Fig 2c).

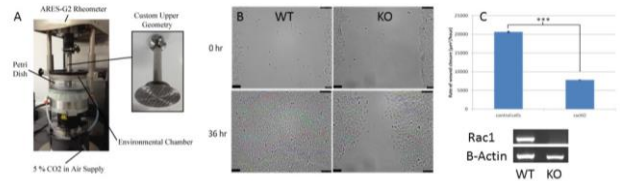


Fig 1: Rheometer customisation (a) with functional and molecular validation of Rac 1 WT and KO cells (b/c). Scale bar bottom left of images, 75µm, ***p<0.001 Mann Whitney U Test.

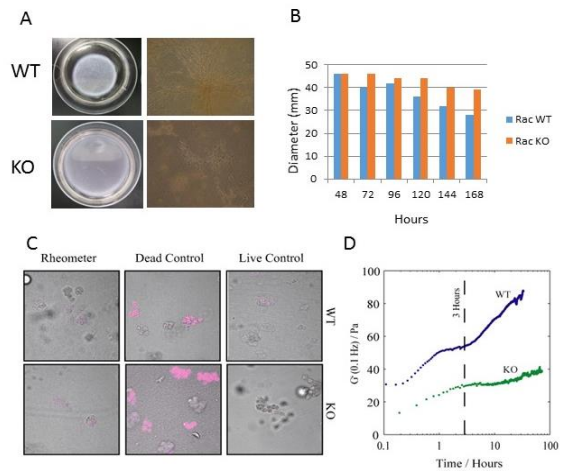


Fig 2: Conventional collagen gel contraction assay after 7 days culture (a) with corresponding daily measurements. Draq7 viability staining of WT and KO cells grown in our customised rheometer with corresponding changes in G prime (d).

DISCUSSION & CONCLUSIONS:

A novel biorheometer for *in vitro* real time monitoring of matrix remodelling has been developed which detected matrix remodelling at earlier time points than conventional methods.

ACKNOWLEDGMENTS:

The authors would like to thank the EPSRC (Grant EP/H45848/1) for providing financial support for this project and Prof Klemens Rottner, University of Bonn for donation of Rac1 WT and KO cells.

Identification of distinct niches of functional mesenchymal stem cells in bone marrow and synovial tissues in a mouse model of joint surface injury.

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INTRODUCTION: We previously identified functional mesenchymal stem cells (MSCs) in the synovium of mouse knee joints¹. Here we investigated the presence of a similar population in bone marrow. As nerve growth factor (NGF) has been reported to be a key regulator of non-inflammatory joint pain² we investigated its expression in an acute articular cartilage injury model.

METHODS: Our double analogue nucleoside labelling scheme¹ and joint surface injury murine model³ were combined in order to label stem cells in vivo. Paraffin sections of knee joints were analysed for the presence of nucleoside-positive cells and expression of MSC markers using double immunofluorescence staining. A time point analysis of NGF expression in articular cartilage was carried out by immunohistochemistry. A Transwell system was used to assess the in vitro chemotactic effect of NGF on MSCs from synovium or bone marrow.

RESULTS: Populations of long-term nucleoside-retaining cells were identified in both synovium and bone marrow. These cells proliferated in response to injury with marked accumulation at 4 days post injury (dpi), especially in the synovium where they contributed to hyperplasia of the membrane. Phenotypic analysis showed compatibility with MSCs (CD44+, PGFR α +, LNGFR+, CD105+, vWF-, CD45-). Interestingly, CD105 was differentially expressed by slow-cycling cells; it was detectable in IdU-positive cells in the bone marrow but not in the synovium. In the bone marrow, IdU/CldU double-positive cells co-localized with CD146 in the blood

vessel wall. In contrast, perivascular IdU-positive cells in the synovial membrane were distinct from CD146-positive cells.

NGF, observed in uninjured cartilage, showed a decreased staining at 2dpi. At 8dpi the staining was comparable with the uninjured control sample; NGF was also present in the repair tissue. In vitro human BM-derived MSCs migrated in response to NGF as chemotactic agent but synovium-derived MSCs did not.

DISCUSSION & CONCLUSIONS: Our results confirmed previous data in the synovium¹ and reported the presence of a similar population of functional MSCs in bone marrow. Differences in marker expression highlight heterogeneity in the joint microenvironment which might reflect distinct role of bone marrow and synovium-derived MSCs in joint homeostasis, remodelling and repair. In addition, the differential response to NGF confirmed that tissue of origin is an important source of variability in the phenotype and activity of MSCs⁴.

REFERENCES: 1. Kurth TB, *et al. Arthritis Rheum* 2011; 63(5):1289-300. 2. McNamee KE *et al. Pain* 2010; 5; 149(2):386-92. 3. Eltawil NM *et al. Osteoarthritis and Cartilage* 2009; 17(6):695-704. 4. DiMarino AM *et al. Frontiers in Immunology* 2013;4 (JUL).

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EGF and PDGF elicit Wnt-independent β -catenin signalling in mesenchymal stromal cells via integrin-linked kinase.

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INTRODUCTION:

Regenerative medicine therapies targeting osteogenic commitment and differentiation of mesenchymal stromal cells (MSCs) to enhance bone growth and repair are urgently required. Stimulators of the Wnt signaling pathway to act as bone anabolics are receiving considerable attention. On binding to its receptor complex, Wnt acts via Disheveled to inhibit GSK3- β , allowing accumulation and nuclear translocation of active β -catenin to drive expression of Wnt-responsive genes. As GSK3- β may also be inhibited by integrin-linked kinase (ILK), which acts downstream of growth factor receptor and integrin activation, we hypothesized that a Wnt ligand-independent mechanism may also exert control over β -catenin activity in MSCs.

METHODS:

Primary MSCs were treated with recombinant EGF and PDGF in the presence and absence of an ILK inhibitor, after which protein was harvested at 24 hours and western blotting performed to determine active β -catenin and pERK levels. In addition, primary MSCs were treated with either type I collagen or an RGD agonist to stimulate integrin activation, with and without ILK inhibition, followed by western blot analysis.

RESULTS:

We demonstrated that both EGF and PDGF increased active β -catenin levels at 24h, mimicking Wnt ligand-induced signaling, and this effect was blocked by ILK inhibition, compared to GAPDH loading controls (Fig. 1A). Direct integrin activation by exposure to type I collagen and RGD peptides also induced stabilization of β -catenin, which again was dependent on ILK activity (Fig. 1 B and C). Furthermore, ILK inhibition prolonged expression of pERK, an early mediator of EGF/PDGF signaling (Fig. 1 A and C).

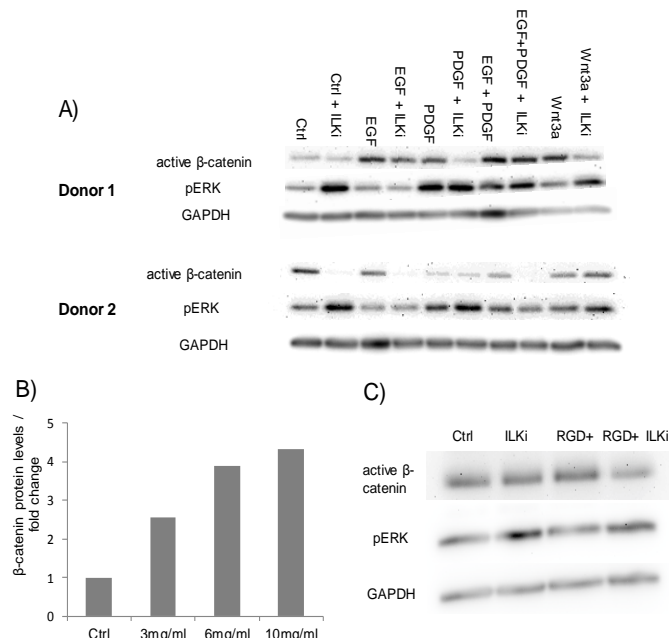


Fig. 1. Active β -catenin and pERK levels in response to PDGF/EGF stimulation (+/- ILK inhibition, ILKi) (A), integrin activation by type I collagen exposure, densitometry analysis (B) and RGD peptide stimulation (C).

DISCUSSION & CONCLUSIONS:

Our evidence indicates that the β -catenin signaling pathway can be independently regulated by growth factor and integrin-mediated adhesive interactions in MSCs, without the requirement for Wnt ligand stimulation. These findings provide a greater understanding of the regulatory cues that control MSC function and reveal previously unrecognized mechanisms to maximize regenerative medicine strategies for skeletal therapeutics.

ACKNOWLEDGMENTS:

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Synthesis and *In Vitro* Biocompatibility of Multi-substituted Hydroxyapatite for Bone Tissue Engineering Applications

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INTRODUCTION:

Synthetic hydroxyapatite (HA) possesses good biocompatibility, bioactivity and osteoconductivity and closely resembles the mineralised phase of human bone and teeth. For many years the clinical researchers worldwide have been aware that the mineral phase of bone contains a number of substituents (*e.g.* CO₃, Si, Zn, Sr, Na, and Mg) and it is not solely calcium phosphate. Carbonate is the major substitute among the trace elements which are present, about 2-8wt% of which varies depending on age. The presence of approximately 2wt% Si is known to be essential in bone formation and calcification¹.

The aim of this study is to synthesise multi-substituted HA with the incorporation of carbonate and silicon ions simultaneously, and to assess the *in vitro* cytotoxicity of the as-synthesised powders in response to human Bone Marrow derived Mesenchymal Stem Cells (hMSCs). It is intended that this novel formulation will improve both osteogenic behaviour and mechanical properties of the resulting scaffolds.

METHODS:

Multi-substituted HA (SiCHA) powders were synthesised at room temperature using Ca(NO₃)₂·4H₂O, (NH₄)₂HPO₄, NH₄HCO₃ and Si(CH₃COO)₄ (Sigma-Aldrich, Gillingham, UK) by nanoemulsion method². The amounts of carbonate (x) and silicon (y) substituted into the HA structure were calculated based on the stoichiometry empirical formula as shown below:
SiCHA: Ca_{10-x/2} (PO₄)_{6-x-y} (CO₃)_x (SiO₄)_y (OH)_{2-y}

The physico-chemical properties of the as-synthesised powders were investigated through XRD, FTIR, CHN and ICP. The biocompatibility of the as-synthesised powders was then tested *in vitro* cell-culture with hMSCs (P2) via direct contact based on ISO10993-5.

RESULTS:

Both the carbonate and silicon ions successfully substituted into the HA lattice. From the physico-chemical characterisations, 2.0CHA, 0.5SiHA, 2.0:0.3 and 2.0:0.5SiCHA were chosen as the optimum powders.

The powders were non-toxic to hMSC cells. Powders with carbonate encouraged faster cell proliferation and produced relatively higher amounts of total protein as they developed in comparison to the carbonate-free powders.

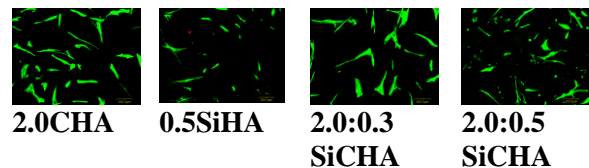


Fig.1. Live/dead images of the optimum powders after 14 days in culture

DISCUSSION & CONCLUSIONS:

The multi-substituted HA (SiCHA) powders with different carbonate to silicate molar ratio were successfully synthesised using a nanoemulsion method. No secondary phases were detected to contaminate the multi-substituted HA. *In vitro* cytotoxicity assessment shows that the majority of cells remained viable after 14 days in culture. The results also showed that the presence of carbonate ions, or carbonate and silicon ions in combination, stimulated faster cell proliferation and greater volumes of protein production.

REFERENCES:

1. Sprio, S. *et al.*, Materials Science and Engineering C28: 179-187. 2008.
2. Zhou, W.Y. *et al.*, Journal Material Science: Material Medicine 19: 103-110. 2008.

ACKNOWLEDGMENTS:

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Enhanced Osteogenesis of hMSC in Hydroxyapatite/Fibrin Gels

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INTRODUCTION:

Hydroxyapatite (HAp) is a calcium phosphate mineral commonly found in bone¹. Fibrin is a naturally occurring polymer responsible for clot formation and orchestration of regenerative processes, such as angiogenesis and osteogenesis². Combination of these materials could enhance tissue regeneration rate and seeding efficiency of implantable constructs for bone tissue engineering.

METHODS:

HAp rod-like, carbonated nanoparticles, Ca/P > 1.67 were synthesised using a controlled sol-gel methodology. In brief, fibrin gels were produced using bovine fibrinogen 0.2mg/ml and thrombin 13.25U/L³. Immortalised hMSC were mixed with the fibrinogen to give 2x10⁵ cells/gel. HAp was dispersed in the thrombin solution to give 500µg/ml final gel concentration. Fibrinogen and thrombin solutions were mixed in a sterile cylinder and polymerised for 1 hour in an incubator. Gels were cultured in basal and osteogenic media for 21 days. Histological differences were observed using Masson's trichrome and osteogenic gene expression was analysed using RT-PCR. Statistical analysis was performed and data represented as mean ± SEM.

RESULTS:

Histology of fibrin sections stained with Masson's trichrome revealed enhanced collagen deposition from cells within the fibrin-HAp matrix. RT-PCR showed an increase in ALP, Runx2, OPN and OC in HAp containing fibrin compared with control.

ACKNOWLEDGMENTS:

The authors would like to acknowledge the support of Arthritis Research UK (Award 19429) and the FP7 RESTORATION project (Award CP-TP 280575-2).

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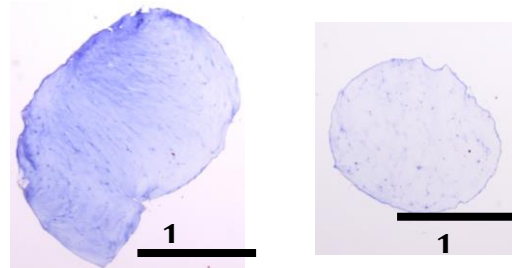


Fig. 1 Section of fibrin-hMSC following 21 days cultivation with osteogenic medium. A) Fibrin & B) Fibrin-HAp.

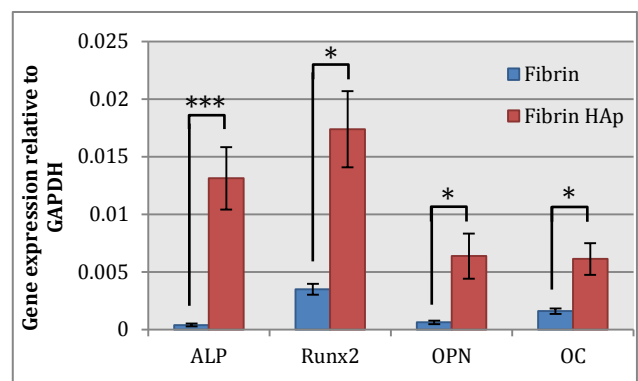


Fig.2. Relative gene expression of RNA isolated from hMSC following 21 days cultivation in osteogenic medium. * $p < 0.05$ & *** $p < 0.001$.

DISCUSSION & CONCLUSIONS:

The properties of HAp and fibrin alone are well documented and should act synergistically for bone regeneration *in-vivo*. This data indicates that combination of these two materials promote hMSC osteogenic differentiation *in-vitro*.

REFERENCES:

1. E. Bouyer et al (2000), J Mater Sci 11:523-531.
2. I. Catelas et al (2006), Tissue Engineering 12: 2385-2396.
3. T. Osathanon et al (2008), Biomaterials 30: 4091-4099.

Electrostatic Stabilisation of Bio-Ink through the Cationic Encapsulation of Cells for Piezo Drop on Demand Inkjet Printing

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INTRODUCTION: Under the remit of tissue Engineering and regenerative medicine, bioprinting is an emerging tool which, it is hoped, will underpin the fabrication of complex tissue structures. Fundamental to inkjet printing is the ink itself. Researchers have found that cell suspensions were prone to settling and agglomeration and have realised no viable solution¹. Electrostatic stabilisation using polyelectrolyte coatings is a method of stabilising pigment in common inkjet inks. Many, if not all of these coatings however, are cytotoxic and thus inappropriate for use in a bioink. A cationic polymer, Poly-L-Lyseen (PLL) is known to be both cell compatible² and an electrostatic stabiliser³. It was therefore hypothesised that encapsulation of cells in a coating of PLL would aide dispersion. The objectives were to ascertain the correct coating thickness as to allow the cell to release from the encapsulant post print and to visually assess the influence of the coating on print efficacy.

METHODS:

Cell Encapsulation: Human osteosarcoma cells (U2OS, Sigma-Aldrich, UK) were cultured in completed Alpha-Modified Eagle's Medium. Three concentrations, 200 $\mu\text{g.ml}^{-1}$, 400 $\mu\text{g.ml}^{-1}$ and 2000 $\mu\text{g.ml}^{-1}$, of Cationic PLL (Sigma) were prepared and each added to 2 million U2OS cells suspended in 1 mL of HBSS. After incubation, washes in HBSS were made before centrifugation and re-suspension. 100 μl of the preparations were transferred into a 12 well plate and the cell response to encapsulation observed.

Bioprinting: 2 milion U2OS cells coated in PLL of concentration 200 $\mu\text{g.ml}^{-1}$ were re-suspended in 2ml HBSS, along with 1x10⁶cell.ml⁻¹ uncoated U2OS cells in 2ml of HBSS, for printing. The cell specimens were loaded into reservoirs of a piezo based, DOD inkjet printing system (Microfab, USA). 100 μl controls were pipetted into 3 wells of a 12 well plate, containing 1ml Eagle's Medium in each well, before the remaining 9 wells received 15k ink drops from a 80 μm orifice.

RESULTS: Viewed after encapsulation, the cells coated in the 200 $\mu\text{g.ml}^{-1}$ PLL exhibited thinner

coatings than those cells coated in the 400 $\mu\text{g.ml}^{-1}$ and 2000 $\mu\text{g.ml}^{-1}$ concentrations. After 7 days the cells encapsulated in the 2000 $\mu\text{g.ml}^{-1}$ and 400 $\mu\text{g.ml}^{-1}$ concentrations were seen to be unattached. Cells coated at 200 $\mu\text{g.ml}^{-1}$ attached to the well plate in the absence of PLL residues. Printing of the unencapsulated cells showed aggregated colonies, from up to 8 colonies in the first well and 0 in the latter. Printing of the encapsulated cells showed well dispersed individuals. Debris was seen in the orifice during printing and, as with the uncoated cells, the first wells to be printed showed higher deposition rates than the latter.

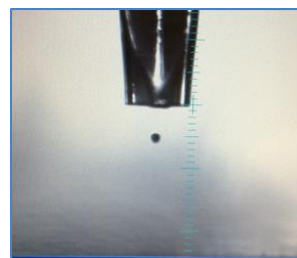


Fig. 1 a single droplet of bio ink with a diameter of 83 μm and 299pl volume

DISCUSSION & CONCLUSIONS:

Preliminary work has suggested that a cationic cell coating aids cell dispersion. Cell deposition rates dropped in both cases. In the instance of unencapsulated cells this was due to cell agglomeration, and in the printing of encapsulated cells was due to fouling of the orifice, presumed to be PLL. These results demonstrate a strong case for further work on the otherwise unexplored concept of printing cells with charged coatings.

REFERENCES:

1. J.Cameron et al (2013), Biomater **1**: 224-230
2. A.Diaspro et al (2002), Langmuir **18**: 5047-5050
3. J.Kang et al (2012), Langmuir **28**: 16751-16760

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Control of Collagen Hydrogel Compression for Cell Rescue

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INTRODUCTION:

During the compression of collagen hydrogels, water or small molecules can freely travel between inter-fibrillar spaces, whereas large molecules, such as oligomeric collagen species, are thought to block the fluid leaving surface (FLS)¹. We hypothesize that artificially incorporated large polymers can block the FLS in a similar fashion, allowing control of gel compression rates. This in turn can reduce any cell damage or excessive particle loss due to high fluid flow rates in some (blended collagen) hydrogels.

METHODS:

Large polymers used within the gel include fibrinogen (340kDa; Sigma-Aldrich) in 0.9% saline, dextran (500kDa; Fisher Bioreagents) or poly(ethylene oxide)(PEG; 400kDa or 1000kDa, Sigma-Aldrich) in deionised water to 0, 2, 5 or 10mg/ml. The polymer solution was mixed 1:9 into neutralised collagen solution (10% 10xMinimum essential media (Gibco, UK) and 80% acid-soluble collagen (Firstlink; UK)) before plating 2.5ml into 24-well plates (37°C incubation, 30 minutes). Hydrogels were compressed using a paper-roll plunger (Whatman grade 1 paper, 95x4cm) at a rate measured as mass (fluid) gained by plungers. To test if cells can be rescued from high fluid shear stress, 10mg/ml PEG was added to gels where 50% of the collagen solution was substituted with polymeric collagen (extracted from calf tendon in 0.5M acetic acid after EDTA treatment)², containing 15,000 human dermal fibroblasts per gel. Cell activity analysed with Alamar blue assays.

RESULTS:

The rate of hydrogel compression is dependent on the concentration and hydrodynamic radius of the added polymer. Polymers <11nm did not affect compression rate. The rate of compression generally decreased with increasing mobile polymer concentration, and 10mg/ml PEG, with largest Stokes radius was

most effective in slowing compression rate. Control hydrogels were fully compressed by 23 minutes; whereas gels containing 10mg/ml fibrinogen, dextran or PEG were fully compressed by 24, 36 or 40 minutes respectively. Seeded fibroblasts were rescued from damage with 10% PEG 400 as indicated by a similar cell metabolism reading compared with control gels, 1 day incubation. Cells in all samples continued to proliferate with culture time.

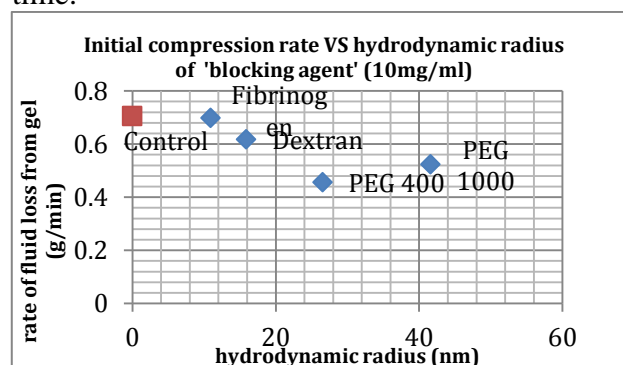


Fig. 1 initial compression rates against polymer hydrodynamic radius

DISCUSSION & CONCLUSIONS:

Compression of blended gels (3 fold increase in modulus) caused significant cell death, but this was reversed by incorporating mobile polymer molecules, such as PEG. This both slowed the fluid flow and spared resident cells. The ability to finely control the extent of plastic compression, hence matrix stiffness, has potential implications for control of cell behavior, and may contribute to increased nanoparticle trapping within gels.

REFERENCES:

1. R. Brown et al (2005) *Adv. Funct. Mater.* **15**: 1762-1770.
2. F. Steven (1967) *Biochim. Biophys. Acta.* **140**: 522-528.

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Cell and Bone Donor age Influence the Osteogenic Activity of MSCs Cultured on an Osteoinductive Washed Human Bone Scaffold

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INTRODUCTION: Bone tissue engineering using native bone scaffolds enables utilization of bones' innate osteogenic abilities, which are not present in synthetic alternatives. However processing techniques required to produce these organic scaffolds (e.g. Gamma irradiation) may weaken their beneficial qualities (e.g. osteoinductivity). Therefore new process methods such as washes are required. Having previously demonstrated the efficacy of a novel bone wash to generate a biocompatible and mechanically stable scaffold¹, this current study aims to assess the washed bones' latent osteoinductive ability. Secondly, given that the majority of live donor allograft bone is obtained from the elderly, the study also aims to assess the effect of cell or bone donor age on mesenchymal stem cell (MSC) osteogenic differentiation and activity.

METHODS: To assess osteoinductive ability, 1cm³ washed human bone cubes were seeded with 5x10⁵ MSCs (N=5), incubated in standard (SM) or osteogenic media (OM) and samples removed at 0, 14 and 28 days for qRT-PCR to assess osteogenic gene expression. Osteogenic activity was assessed by culturing cells from donors <50 and >70 yrs (both N=3) on bone from donors <50 and >70 yrs (both N=3) and analysing gene expression, alkaline phosphatase (ALP) / DNA activity and alamarBlue (AB) metabolic activity.

RESULTS: All samples showed significant (p<0.05) increases in metabolic activity after 14 and 28 days. Culture of MSCs on washed human bone cubes resulted in significant increases in expression of osteogenic markers runx2, osteopontin (OPN) and osteocalcin (OC) (fig 1) at day 14 and OPN and OC at day 28 for both standard and osteogenic media. Assessment of effect of MSC donor age

showed statistically higher gene expression in young cell samples compared to old at day 28 when cultured on bone of varied ages. Interestingly data also showed that old bone induced significantly greater increases in osteogenic gene expression in MSCs compared to young bone; although ALP activity was significantly increased in all samples.

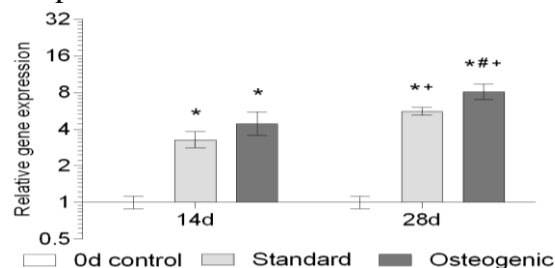


Fig 1. Relative gene expression of OC at 14 and 28 days compared to day 0 in SM and OM. Significances: * = p<0.05 to day 0, + = p<0.05 to day 14 and # = p<0.05 between conditions.

DISCUSSION & CONCLUSIONS: The results of this study demonstrate that the washed biological bone scaffolds are capable of inducing osteogenic differentiation in MSCs independent of osteogenic media. The study further highlights the influence of cell and tissue donor age, with younger cells better able to osteogenically differentiate on the washed bone, and with older bone better able to promote osteogenic activity. These results suggest that whilst using old donor bone is clinically suitable, old donor cells may require stimulatory factors to boost osteogenic activity.

REFERENCES: 1.C.Smith et al (2013), *EurCellMater* 26:7 p83

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Hydrogels from demineralized and decellularized bone extracellular matrix for bone regeneration

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INTRODUCTION:

Treatment of bone defects often involves the use of bone graft substitutes, such as demineralized bone matrix (DBM). Current delivery of DBM particles requires incorporation within a carrier liquid. However, differences in osteogenic activity and inflammation have been reported with various carriers. The objectives of this study were to produce hydrogel forms of DBM and ECM from bovine bone, denoted bDBM and bECM, and to determine their suitability for use in bone regeneration applications.

METHODS:

Bovine femurs were treated with in-house developed protocols¹; resultant materials were pepsin digested and solubilized. Gelation was induced by salt and pH neutralization followed by warming to 37°C. In vitro cell proliferation of mouse primary calvarial cells (mPCs) on the surface of hydrogels was assessed using the CellTiter 96® MTS colorimetric assay and tested for normality and statistically compared using a Tukey-Kramer multiple comparisons test. A 2% gel solution of low viscosity alginate was utilised to prepare alginate and bECM/alginate hydrogels. These were subcutaneously implanted within MF1 nu/nu male mice. After 28 days, the implants were harvested, fixed in 4% PFA, sectioned and stained with Alcian blue/Sirius red.

RESULTS:

Enhanced proliferation of mouse primary calvarial cells was achieved on ECM hydrogels, compared to collagen type I and DBM hydrogels (Figure 1A). Alcian blue staining indicated the presence of cartilaginous material in both alginate and alginate/bECM subcutaneous implants (Figure 1B and 1C) however collagen production (labelled by Sirius red) only occurred in the alginate/bECM implant.

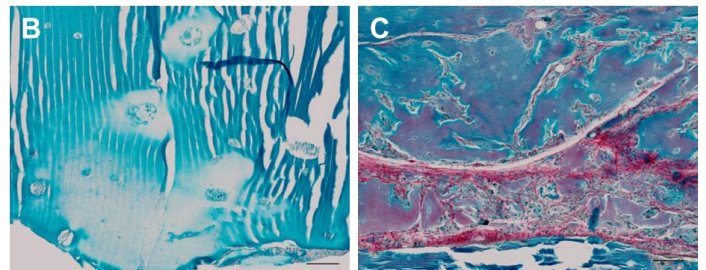
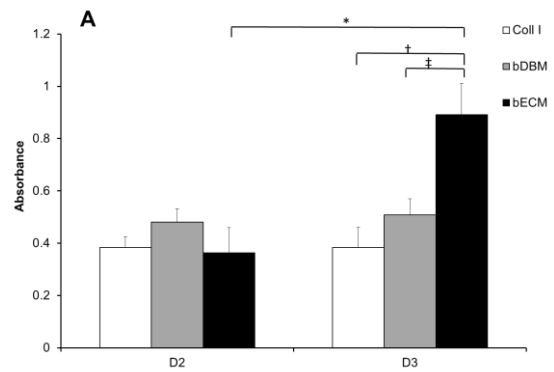


Fig. 1 Proliferation of mPCs on Collagen I, bDBM and bECM hydrogels. * denotes significance between D2 and D3 for bECM, † denotes significance between bECM and Coll I, ‡ denotes significance between bECM and bDBM $p < 0.001$, (A); Alcian blue/Sirius red staining of subcutaneous implant of alginate hydrogel (B) and alginate/bECM hydrogel (C).

DISCUSSION & CONCLUSIONS:

The bDBM and bECM hydrogels possess distinct structural, mechanical and biological properties, including osteogenic functionality. Limited tissue invasion of the alginate hydrogel occurred in the subcutaneous implant whereas the alginate/bECM hydrogel exhibited extensive host tissue invasion and potential for use in bone regeneration.

REFERENCES:

1. M. J. Sawkins et al (2013), Acta Biomater 9: 7865-7873.

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Investigation of potential osteogenesis influence of carbon based nanomaterials

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INTRODUCTION: The unique electronic, mechanical and chemical properties of carbon-based nanomaterials hold great potential in the field of regenerative medicine. Whilst carbon nanotubes (CNTs) have been synthesized using several novel techniques, the methods by which graphene is fabricated have seen dramatic progress in recent years. Whether carbon-based nanomaterials can induce or enhance bone formation and the possible associated mechanisms for such enhancement have not yet been fully investigated. The aims of this study are to assess the potential osteogenic influences of CNTs and two types of graphene oxide on a bone cell line culture and to investigate the possible underlying mechanism.

METHODS: CNTs were produced using chemical vapour deposition (CVD) with methane at 700°C on a bio-silica base using Fe(NO₃)₃·9H₂O as the catalyzer. Both types of graphene oxides, A and B respectively, have also been used. The human bone cell line, MG63, was used for all experiments. 20,000 cells per sample were seeded with α -MEM media supplement with only antibiotic and FCS (10%) for 10 days. In one experimental set-up, CNTs and graphene particles were mixed with poly(lactic acid) (PLA) solution in 1% concentration to form a composite film. 0.5x 1x0.0003 cm film sections were inserted into silicone frames for cell culturing. In the second experimental set-up, cells were cultured in 24-well suspension culture plates with above media incubated with the carbon particles (conditioned media, CM). The media was changed every 2 days. The cell morphology was monitored by optical microscopy every other day. After 10 days in culture, the cells were fixed by 10% formalin.

The fixed samples were then stained by Alizarin Red (ARS) and von Kossa.

RESULTS: Cell aggregates or nodules formed after 2 days in culture in all cases (Fig 1). ARS and von Kossa staining showed stronger minerals deposition (Fig 2) on both the carbon/PLA composite film samples and the samples cultured using CM when compared to the samples cultured on PLA film or using non-conditioned culture media.

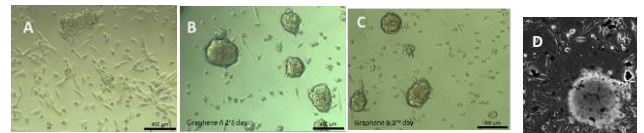


Fig.1 Microscopic images of live cells; A: in non-conditional culture media; B: in graphene A CM; C: in CNT CM; D: on PLA-CNT composite.

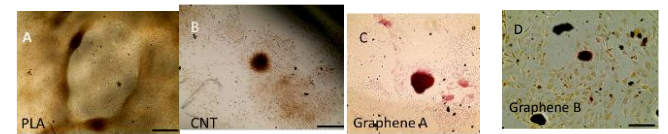


Fig.2 ARS staining images for MG63 grown on PLA film; B: PLA-CNT; C: PLA-graphene A; D: PLA-graphene B. Scale bar is 500 μ m.

DISCUSSION & CONCLUSIONS: The formation of bone nodules in the presence of carbon particles without osteogenic supplemented media within a short culture period indicates that carbon nanomaterials have the capacity to induce bone formation. The carbon particles are suspected to have become the centers which induce cellular aggregation and promote bone nodule formation, do not promote proliferation.

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