Welcome to

The TCES 2021 Virtual Conference

Edinburgh, Scotland (UK)

6 - 7 July 2021

Welcome

It is my pleasure to welcome you to the 20th Annual Meeting of the TCES being held virtually from Edinburgh Scotland. This follows on from a very successful joint TCES-UKSB event hosted by Dr Lisa White in the lovely surroundings of Nottingham, in June 2018. We had hoped to stage our event in the beautiful city of Edinburgh with its iconic landmarks at this special time of year with long evenings and pleasant weather. It became very clear in March 2020 that this was no longer tenable due to the COVID-19 pandemic and we took the heart-breaking decision to cancel the in-person event. It is a testimony to the quality of the speakers and the associated technical programme, that there has been such an encouraging level of interest in this year's virtual conference. We are very grateful to them for their willingness to engage with the new IT-intensive arrangements.

As we have come closer to the date of the event and many of us have endured months of self-isolation and working from home, the value of providing a community event to researchers has become clearer. Recent developments across the field are still pushing forward despite the large hurdles we all have had to face in the past year. We have worked hard to identify a pool of internationally recognised keynote speakers, and extended an invitation to the authors of 30 submitted abstracts to present their work across virtual podium presentations, in both standard presentation format and "turbo" talks. We also have a vibrant poster section, which has been instituted to highlight the exceptional work being done, with a delivery approach that encourages lively discussion. The posters included focus on recent developments in the fields of musculoskeletal, cardiovascular, clinically-driven challenges and developmental models, providing a vibrant and enlighten environment for all.

I would like to thank our Local Organising Committee and the TCES Committee for their help with ensuring a varied and topical programme and chairing the sessions. I thank our enthusiastic and hardworking team of moderators for their tremendous effort in the preparation and daily organisation of this event.

We are all grateful to our sponsors for generous funding, active participation in the sessions and contribution of talks. Special thanks go to our core organisational team of Diane Reid, Emily Martin, Katrina Saridakis, Eddie Dubourg, Laura Smith and Karen Brocklehurst and without whose hard work, strong IT and organisational skills the event would not have been possible.

Anthony Callanan
TCES Organising Chair
The University of Edinburgh

Local Organising Committee:
Dr Anthony Callanan
Prof David Hay
Dr Pierre Bagnaninchi
Dr Jennifer Paxton
Dr Elaine Emmerson

Day 1 – Tuesday, 6th July

12:00 – 12:10 TCES Virtual Conference – Welcome Day 1

12:10 – 13:30 SESSION 1 – Musculoskeletal Tissue Engineering
Chair Professor Sarah Cartmell, University of Manchester

Sponsor Talk - **Jellagen**
Keynote - Professor Fergal O'Brien
Gene Activated Scaffolds for Enhanced Tissue Repair

Paul Humphreys, University of Manchester
Optogenetic Control of BMP Signalling to Drive HPSC Chondrogenesis

Hannah Donnelly, University of Glasgow
A bioengineered bone marrow niche model to support long-term HSCs in vitro

James Armstrong, Imperial College London
Engineering Osteochondral Tissue Gradients

Tosca Roncada, University of Portsmouth
Collagen and alginate hydrogels support re-differentiation of in vitro dedifferentiated ovine chondrocytes

13:30 – 13:40 10 minute - Break

13:40 – 15:00 SESSION 2 - Turbo Talks I
Chair Dr Jennifer Paxton, University of Edinburgh

Sponsor Talk: **BioTek**
Keynote - Professor Kareen Coulombe
Customized Biomaterials for Heart Regeneration with Engineered Myocardium

Bowen Xie, University of Keele
Characterization of foam cell models using a label-free technique toward better atherosclerosis investigation

Annabelle Fricker, University of Sheffield
Tissue engineered cardiac patches for the treatment of post-MI heart failure using natural polymers and human iPSC-derived cells

Marlene Polleres, University of Manchester
A neurovascular 3D cell model to investigate the role of pericytes in dementia

Yunxi Gao, University of Edinburgh
Topographically Featured PCL Electrospun Scaffolds Incorporating Rat Liver Extracellular Matrix (ECM) for Liver Tissue Engineering

Maria Luisa Hernandez Miranda, University of Southampton
The thickness of soft hydrogel substrates modifies bone marrow stromal cell morphology and differentiation

Paola Sanjuan Alberte, University of Nottingham
3D bioprinting of conductive extracellular matrix structures towards cardiac tissue engineering

Melissa Rayner, University College London
Engineered neural tissue made using clinical-grade human neural stem cells supports regeneration in a critical gap-length nerve injury.

Priyanka Gupta, The University of Surrey
Chemotherapeutic Assessment on a Novel Scaffold Assisted Multicellular Model of Pancreatic Cancer

15:00 – 15:15 15 minute – Break

15:15 – 15:45 **SESSION 3 – Poster Session I**

Wendy Balestri, Development of a triphasic 3D in vitro model for the regeneration of the bone-tendon-muscle interfaces
Fer Velazquez de la Paz, PCLMA & PGSM polyHIPE scaffolds for osteochondral regeneration
Albert Ginjaume MSc, Development and characterisation of self-assembling peptide hydrogels as bioinks for 3D bioprinting applications
Miruna Verdes, The influence of fibrous scaffolds in electrical stimulation
Gabrielle Wishart, Radiation Resistance Screening in a 3D Biomimetic Pancreatic Cancer Platform
Annamarija Raic, A new technology for the generation of perfect cell spheroids for cell-based assays
Andra-Maria Ionescu, Identification and isolation of progenitor cell subpopulations within the adult nucleus pulposus: implications for intervertebral disc regeneration
Araida Hidalgo-Bastida, Biosafety of graphene oxide in an in-vivo murine model
Didi Love, Studying the Localisation of Nuclear Lamina Proteins in Human Pluripotent Stem Cell-derived Motor Neurons
Noor Zaini, Identifying mechanical loading parameters to study the mechanobiology of herniation using a computational model
Sara Memarpour Hobbi, Evaluation of nerve-derived extracellular matrix for peripheral nerve regeneration
Matthew Kibble, 3D Bioprinting Whole Intervertebral Discs To Understand Development & Inform Regenerative Therapies
Grace M, 3D Bioprinting Tissue Engineered Meniscal Constructs
Ryan Dimmock, Facile Techniques to Induce Controllable Topographic Patterns on PDMS for Epithelial Tissue Growth
Georgina Al-Badri, Towards A Computational Model Of Vascular Network Formation In A Hydrogel To Aid The Fabrication Of Engineered Tissues
Lorna Westwood, Incorporation of Retinyl Acetate into PCL Electrospun Scaffolds for Antioxidant Delivery
Tina Dale, Reduced oxygen culture promotes proliferation and a mucin-producing phenotype in COPD distal airway stem cells
Ella-Louise Handley, Electrospun PCL-Ascorbic Acid Fibres as Antioxidant Cardiac Tissue Scaffolds, Ella-Louise Handley & Anthony Callanan
Nancy Hussein, Dental Tissue Engineering Using Diabetic Bone Marrow Mesenchymal Stromal Cells
Jenny Katsouli, Identifying and characterizing the phenotype of cells for use in peripheral nerve tissue engineering
Samuel Higginbotham, Investigating how secreted factors from adipose tissue alter myofibroblast and scar phenotype
Miguel Ferreira, Human Cartilage Developmental Model Using Embryonic Stem Cells Cultured Within 3D Hydrogels
Thomas Carvell, Single step, centrifugation-free cell washing and medium exchange for cellular therapeutics
Bowen Xie, Characterization of foam cell models using a label-free technique toward better atherosclerosis investigation
Melissa Rayner, Engineered neural tissue made using clinical-grade human neural stem cells supports regeneration in a critical gap-length nerve injury
Maria Luisa Hernandez Miranda, The thickness of soft hydrogel substrates modifies bone marrow stromal cell morphology and differentiation
Paola Sanjuan Alberte, 3D bioprinting of conductive extracellular matrix structures towards cardiac tissue engineering
Priyanka Gupta, Chemotherapeutic assessment on a novel scaffold assisted multicellular model of pancreatic cancer
Marlene Polleres, A neurovascular 3D cell model to investigate the role of pericytes in dementia
Annabelle Fricker, Tissue engineered cardiac patches for the treatment of post-MI heart failure using natural polymers and human iPSC-derived cells
Lydia Beeken, Assessment of the Therapeutic Potential of Corneal Mesenchymal Stem Cells for Ocular Surface Disorders

15:45 – 17:35 SESSION 4 - Robert Brown Early Stage Investigator
Chair Dr Nicholas Evans, University of Southampton

Sponsored by: **PeproTech**
Keynote - Professor Molly Stevens
New materials based strategies for regenerative medicine

George Bullock, University of Sheffield
The mechanisms of soft tissue toxicity in medication-related osteonecrosis of the jaw

Kenny Man, University of Birmingham
Epigenetic reprogramming promotes the therapeutic potency of osteoblast-derived extracellular vesicles for bone regeneration

Keynote - Professor Brendan Harley
Building hierarchy: engineering porous scaffolds for regenerative medicine

17:35 – 18:00 Table-Top Quiz – Prize to be awarded at end of Virtual Conference Day 2

Day 2 – Wednesday 7th July

11:30 – 12:00 TCES – Annual General Meeting: Open to all members and registrants to attend

12:00 – 12:10 TCES Virtual Conference – Welcome Day 2

12:10 – 13:30 SESSION 5 – New Frontiers Tissue Engineering
Chair Dr Vanessa Hearnden, University of Sheffield

Sponsor Talk: **Cellink**
Keynote – Professor Liesbet Geris
Digital twins of living implants: applications in osteochondral tissue engineering.

Sílvia Paiva, Royal College of Surgeons in Ireland
Bio-instructive Collagen-Magnesium Nanocomposite Scaffolds to Manipulate Bone Metabolism and Promote Bone Repair

Samuel Moxon, University of Manchester
A 3D Bioprinted Model of the Human Intervertebral Disc

Shivesh Anand, Maastricht University
Role of Scaffold Geometry in Tympanic Membrane Tissue Engineering

Roxanna Ramnarine, University of Southampton
Self-assembling Nanoclay gels with 3D Micropatterning of BMP-2 for Bone Tissue Regeneration

13:30 – 13:40 10 minute - Break

13:40 – 15:00 SESSION 6 - Turbo Talks II
Chair Dr Elaine Emmerson, University of Edinburgh

Sponsor Talk: **Fluicell**
Keynote – Dr Christine Horejs
Scientific writing and publishing – insights from a Nature editor

Jonathan Hinchliffe, University of Sheffield
Semi-artificial pancreas for the treatment of Type 1 diabetes: Perspectives, challenges, and solutions

Roxana Sava, University of Manchester
Developing a Hydrogel-assisted Combination Therapy for Brain Repair Following Ischaemic Stroke

Alessandra Grillo, University College London
Bioengineering a 3D tissue model of the rectus sheath to study herniation

Busra Baskapan, University of Edinburgh
Elucidating the effects of varying laminin emulsions in Polycaprolactone electrospun fibres

Lydia Beeken, University of Nottingham
Assessment of the Therapeutic Potential of Corneal Mesenchymal Stem Cells for Ocular Surface Disorders

Verónica Hidalgo Álvarez, Heriot-Watt University
Single-Administration Vaccine Enhancement (SAVE).

Matt Shephard, University of Keele
Functionalised Nanosurfaces for Animal-Product Free 3D-Substrates

Krit Rattanawonsakul, University of Sheffield
The effect of Geranylgeraniol (GGOH) on zoledronate-induced toxicity on oral fibroblasts and keratinocytes.

15:00 – 15:15 15 minute – Break

15:15 – 15:45 SESSION 7 – Poster Session II

Vibin Alageswaran, Cellular response to substrate topography, chemistry and cyclical loading
Sara Barreto, Investigation of the Volatilome of Stem Cells using Selected Ion Flow Tube Mass Spectrometry
Syed Mohammad Daniel Syed Mohamed, Kidney Tissue Engineering using Polyhydroxyalkanoates
Auxtine Micalet, Contractility signatures of cancer cells using 3D in vitro models
Amy Harding, Utilising human tissue-engineered skin equivalents to determine chemical irritation potential
Alex Sturtivant, Investigating the use of single material 'composite' alginate scaffolds for cartilage tissue engineering
Idris S Kalokoh, Using nitric Oxide as a reliable marker to detect macrophage' activation state
Flavia Carton, Hydrogels characterization for Skeletal Muscle Regeneration
Sophia Read, Bioprinting the Hierarchical Extracellular Matrix Environment for Articular Cartilage Repair
Tania Choreno Machain, Low-pressure oxygen plasma treatment as a straightforward method for bioactivity enhancement in-vitro of a polyethylene terephthalate anterior cruciate ligament scaffold
Amy Byrne, Tenocyte driven immunomdulation
Jennifer Paxton, Manufacturing 3D co-cultures of the bone-tendon interface using human anatomical morphometrics
Vinothini Prabhakaran, Towards scaffold-less bone production: investigating spheroid formation in rat osteoblasts
Nur Rofiqoh Eviana Putri, Additive Manufacture of Vascularised Hybrid Scaffolds for Bone Tissue Engineering
Krishna Patel, Microfluidic Development of Microparticles to Enhance Cell Engraftment
Christina Loukopoulou, Modifying bone scaffold characteristics in an anatomical interfacial tissue engineered model
José Rui Oliveira Rodrigues, Serum-free Osteogenic Differentiation of Mesenchymal Stem Cell Line
Jason Hutchinson, Microcarriers with Complex Architectures Manufactured by Two-Photon Lithography for Mechanobiological Manipulation and Expansion of Mesenchymal Stem Cells
Ami Nash, Novel formulations for the spray delivery of therapeutic cells for traumatic brain injury
Denata Syla, Expansion of Human Mesenchymal Stem Cells under Serum-Free Conditions
Sarah Dallas, Using nanoscale mechanotransduction to stimulate differentiation of neural stem cells and gliobasltoma-derived cancer stem cells
Amara Freed, Bone and Tissue Engineering of the Temporomandibular Joint (condyle): A Histological Investigation of the Rat Condyle, Knee and Hip
Jonathan Hinchliffe, Semi-artificial pancreas for the treatment of Type 1 diabetes: Perspectives, challenges, and solutions
Verónica Hidalgo Álvarez, Single-Administration Vaccine Enhancement (SAVE)
Krit Rattanawonsakul, The effect of Geranylgeraniol (GGOH) on zoledronate-induced toxicity on oral fibroblasts and keratinocytes
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Busra Baskapan, Elucidating the effects of varying laminin emulsions in Polycaprolactone electrospun fibres
Roxanna Ramnarine, Self-assembling Nanoclay gels with 3D Micropatterning of BMP-2 for Bone Tissue Regeneration

15:45 – 17:05 SESSION 8 - Clinically-Driven & Translational TE: Challenges and Opportunities
Chair Professor James Phillips, University College London

Sponsor Talk: **Catapult Cell and gene Therapy**
Keynote – Professor Jonathan Fallowfield
Drug development for non-alcoholic fatty liver disease

Laura Sidney, University of Nottingham
A novel preservation technique for the manufacture of biocompatible and terminally sterilised transplantable human corneas

Tom Bate, University of Edinburgh
Comparison of donor variation in human liver ECM-PCL electrospun scaffolds

Trisha Vikranth, University of Keele
Decellularised pleural membrane patches for prolonged alveolar air leaks

Sarah Shafaat, University of Sheffield
Developing an estradiol-17ß [E2] responsive tissue engineered vaginal tissue model for evaluating biomaterials to be used in the female pelvic floor repair

17:05 – 17:15 10 minute – Break

17:15 – 17:45 Prizes & Close of TCES Virtual Conference 2021

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Session Chairs

Professor Sarah Cartmell;
Professor of Bioengineering

Sarah's interdisciplinary research area focuses on creating a paradigm shift in healthcare treatments. Her research is in the area of orthopaedic tissue engineering, wound care treatments and more recently, translating the 3D tissue growth techniques to cancer research for early biomarker detection. Her research involves developing biomaterials and stimuli such as mechanical or electrical stimulation regimes to grow bone, cartilage, tendon and ligament tissue in the laboratory with the aim of potentially implanting these tissues into a patient. In order to assess the quality of the tissues, she also develops X-ray imaging techniques for dynamic soft tissues or live cell imaging. She is currently Head of the Department of Materials which is home to nearly 2,000 students and staff and Director of the EPSRC Centre for Doctoral Training for Advanced Biomedical Materials.

Elaine Emmerson

Elaine graduated from The University of Liverpool in 2004 with a B.Sc. (Hons) in Genetics. Following that she worked as a research technician at the University of Manchester, before beginning a Ph.D. in wound healing in 2006, receiving her doctorate in 2010. Following this, Elaine worked for 2 years as a post-doctoral research associate, investigating the effect of ageing on cutaneous wound healing and how estrogen therapy elicits positive effects in postmenopausal women. In 2013 Elaine moved to the University of California San Francisco to begin a Postdoctoral Research Fellow position. During this time she investigated the interaction between nerves and stem cells during organ development, using the mouse and human salivary gland as a model organ. Following this Elaine took the knowledge she had learned from her developmental studies and applied it to better understand regeneration of the adult salivary gland. In 2017 she was awarded a Chancellor's Fellowship to initiate her independent research group within The Centre for Regenerative Medicine at The University of Edinburgh, to study salivary gland regeneration following head and neck radiotherapy. In February 2018 she was awarded a UK Regenerative Medicine Platform Innovation Award to develop therapeutics to regenerate injured salivary gland in head and neck cancer patients. More recently she has been awarded funding from the Medical Research Council, Wellcome Trust, Tenovus Scotland and The Royal Society to further investigate the cellular communication that occurs during salivary gland regeneration.

Dr Vanessa Hearnden
Lecturer in Biomaterials and Tissue Engineering
University of Sheffield

Vanessa Hearnden joined the Department of Materials Science and Engineering in 2015 as Lecturer in Biomaterials and Tissue Engineering. Vanessa's undergraduate degree was in Biomedical Science; she then obtained her PhD in Tissue Engineering from the University of Sheffield in 2011. She has previously worked as a post doc in the Medical School and School of Clinical Dentistry, gaining experience of both lab-based studies and clinical research. Vanessa serves as Chair of the White Rose Biomaterials and Tissue Engineering Group (BiTEG) and on the committee for the Tissue and Cell Engineering Society (TCES). Vanessa's research focuses on tissue engineering as a tool to both understand the fundamentals of disease processes and to develop novel diagnostic and treatment strategies. With expertise in primary cell biology and three-dimensional cell biology, Vanessa's work focuses on developing regenerative medicine strategies to improve soft tissue wound healing.

Nick Evans

Nick Evans was appointed as a lecturer in Bioengineering at Southampton University in January 2011. Before his appointment, Nick completed his PhD at King's College under the supervision of Prof John Pickup, where he researched novel techniques in fluorescence spectroscopy for tracking metabolism in cells by using their natural fluorescence. After experiencing some of the excitement of stem cell biology during his PhD studies, he won an MRC career development fellowship at Imperial College to research the effects of extracellular matrix on the differentiation of embryonic stem cells. He then took a postdoctoral position at Stanford University to study Wnt signalling and stem cells in wound healing, and was subsequently awarded a SPARK fellowship in translational medicine to develop therapies for stimulating skin regeneration. Nick is now an associate professor at the University of Southampton and his current research focuses on drug delivery biomaterials, and biomechanics in tissue healing and regeneration.

Dr Jennifer Z Paxton
Senior Lecturer in Anatomy
University of Edinburgh

Jennifer graduated in Anatomy from the University of Glasgow in 2004 and then studied for an MSc Bioengineering at the University of Strathclyde. Here, she combined her love of anatomy with engineering to understand the ways it can be applied to healthcare. It was here that Jennifer discovered the Tissue Engineering field and so moved to the University of Dundee and completed her PhD in 2009. Jennifer then undertook a period of postdoctoral research (2009-2013) in Chemical Engineering at the University of Birmingham before joining the University of Edinburgh in 2014 as a Lecturer in Anatomy, being promoted to Senior Lecturer in 2020. Here, her lab focusses on the repair/regeneration of hard-soft tissue interfaces and is funded by Orthopaedic research UK, Medical Research Scotland, Tenovus Scotland, The Carnegie Trust and the Anatomical Society. She also contributes to a wide range of anatomy teaching across medical and science programmes, including establishing the first online postgraduate diploma in Anatomical Sciences.

Jennifer is also a keen science communicator and regularly engages with the public and schools. She has won several awards for science communication and also holds a Royal Society Partnership Grant with a local primary school. She has also recently published 3 public interest anatomy books.

James Phillips

James Phillips is Professor of Regenerative Medicine and Vice-Dean (Innovation & Enterprise) in the Faulty of Life Sciences at University College London. He is also co-Director of the UCL Centre for Nerve Engineering and Chief Scientific Officer of the UCL spinout company Glialign Ltd. His research focus is on translational neuroscience including construction of living artificial tissues for regenerative medicine, developing novel cell, drug, gene and biomaterial therapies for neural repair and protection, and construction of advanced 3D co-culture models. Applications include treating and modelling neurodegenerative diseases and traumatic injury to peripheral nerves, the spinal cord and the brain. His multi-disciplinary research group is based in the Department of Pharmacology at the UCL School of Pharmacy, and uses in silico, in vitro, in vivo and clinical approaches.

Keynote Speakers

Liesbet Geris

Professor in Biomechanics and Computational Tissue Engineering

University of Liège and KU Leuven, Belgium

Digital twins of living implants: applications in osteochondral tissue engineering.

Synopsis: Digital Twin is a classical engineering concept referring to a virtual representation (in silico model) of a physical object or system across its lifecycle. In healthcare, the term is used more loosely to refer to a personalized in silico model. The underlying model technology ranges from purely data-driven (bioinformatics, AI) to mechanistic models, depending on the available knowledge and the question to be answered. In this lecture I will discuss a number of digital twins developed in the context of osteochondral TE. Models focusing on the cellular part of the TE product look into predicting and optimizing differentiation of patient-derived progenitor cells, using a skeletal cell atlas as a reference. Biomaterial models assist in the design and printing of 3D scaffolds. The digital twin of an in-house developed perfusion bioreactor system allows to interpret, interpolate and enhance the in-line sensor read-outs. Finally, models of the in vivo processes focus on understanding the biological complexity and predicting effects of therapeutic strategies in the patient. These examples will demonstrate how digital twins in TE contribute to making the bridge between in vitro and in vivo processes and thereby assist in the translation from the laboratory to the patient.

Bio:

Liesbet Geris is Professor in Biomechanics and Computational Tissue Engineering at the university of Liège and KU Leuven in Belgium. Her research focusses on the multi-scale and multi-physics modeling of biological processes. Together with her team and their clinical and industrial collaborators, she uses these models to investigate the etiology of non-healing fractures, to design in silico potential cell-based treatment strategies and to optimize manufacturing processes of these tissue engineering constructs. Liesbet is scientific coordinator of the Prometheus platform for Skeletal Tissue Engineering (50+ researchers). She has edited several books on computational modeling and tissue engineering. She has received 2 prestigious ERC grants (starting in 2011 and consolidator in 2017) to finance her research and has received a number of young investigator and research awards from the in silico and regenerative medicine communities. She is a former member and chair of the Young Academy of Belgium (Flanders) and member of the strategic alliance committee of the Tissue Engineering and Regenerative Medicine Society. She is the current executive director of the Virtual Physiological Human Institute and in that capacity she advocates the use of in silico modeling in healthcare through liaising with the clinical community, the European Commission and Parliament, regulatory agencies (EMA, FDA) and various other stakeholders. Besides her research work, she is often invited to give public lectures on the challenges of interdisciplinarity in research, women in academia and digital healthcare.

Kareen L.K. Coulombe, PhD

Associate Professor of Engineering
Center for Biomedical Engineering
Brown University, Providence, RI, USA

Customized Biomaterials for Heart Regeneration with Engineered Myocardium

Coronary heart disease and heart failure are major contributors to the global burden of cardiovascular disease, yet novel therapeutics to replace the loss of cardiac contractility are critically needed. We aim to restore contractile function to the heart after myocardial infarction (MI) using tissue engineered from human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes with strategies for implant integration. Using customized biomaterials, we design implants with the goal of remuscularizing the ventricular wall and revascularizing the infarct and implant. Our human engineered cardiac tissue integrates the biomechanics of the scaffold architecture with the maturing contractile phenotype of hiPSC-derived cardiomyocytes using natural biomaterial systems including wet-spun collagen microfibers in bespoke anisotropic architectures. We leverage the innate ability of the host vasculature to grow into implanted engineered tissue by using customized alginate biomaterials for controlled release of angiogenic factors to instruct new vessel growth into implanted engineered cardiac tissue in a rat ischemia/reperfusion MI model. Our results show improved 3D vascular perfusion of the engineered human cardiac tissue and improved whole heart function. Ongoing work integrates polycaprolactone for advanced structural control and customized composite alginate hydrogels for improved biologics retention and release.

Bio:

Dr. Coulombe earned a B.S. in Biomedical Engineering at the University of Rochester summa cum laude in 2001 and was a Whitaker Pre-doctoral Fellow, earning a Ph.D. in Bioengineering at the University of Washington in 2007. She was an NIH Ruth L. Kirschstein post-doctoral fellow in Pathology at the University of Washington where she won an NIH Pathway to Independence K99/R00 award in 2012, and she started as an Assistant Professor of Engineering and Medical Science at Brown University in January 2014. She was inducted into the Athletic Hall of Fame at the University of Rochester in 2016; was named a Rising Star in 2017 by the Cellular and Molecular Bioengineering Group of BMES; was a Finalist for the Young Investigator Award at the Regenerative Medicine Workshop at Charleston (2019); and was awarded a 2021 NSF CAREER Award. She lives in Pawtucket, RI with her husband and two daughters, and enjoys creative outlets and being in nature.

Professor Jonathan Fallowfield

Personal Chair of Translational Liver Research, University of Edinburgh

Drug development for non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a complex disease with a growing prevalence and costly impact on society. NAFLD represents a spectrum of disease from isolated fatty liver (steatosis) through to the progressive, inflammatory form – non-alcoholic steatohepatitis (NASH) – and potentially incremental liver scarring (fibrosis) that may culminate in end-stage scarring (cirrhosis) and a risk of liver cancer and premature death. However, disease progression is highly variable and unpredictable, with no qualified non-invasive biomarkers. Although weight loss >10% can reverse NASH and fibrosis, this is rarely achieved or sustained. To date, there are no licensed pharmacological therapies for NASH despite the growing unmet medical need. Drug development in NASH is challenging and the so-called 'NASH graveyard' is littered with a growing number of failed clinical trials and terminated drug programs. These disappointments can, at least in part, be attributed to a lack of physiologically relevant and predictive preclinical NAFLD/ NASH models that translate to humans. Indeed, successfully modelling all of the key pathogenic events (from steatosis to inflammation and fibrosis) evident in clinical NASH has been extremely challenging – in both in vivo animal models and in vitro cell based ones. Additionally, it seems unlikely that a single, 'one-size-fits-all' wonder drug will be discovered for NASH. Many pharmaceutical companies are therefore shifting their R&D efforts toward combination therapies.

In this talk, I will discuss how sophisticated bioengineering models, incorporating multiple human liver cell types along with complex biochemical and biomechanical micro-environments, may be used to better mimic human in vivo pathophysiology and help identify drug candidates with improved safety and efficacy profiles to advance to clinical trials. Increasingly, computational approaches, leveraging human big data resources, can be used to identify the most appropriate and translationally relevant compounds and drug combinations which target identified pathways involved in human NAFLD/NASH.

Bio:
Professor Fallowfield is a Principal Investigator in the Centre for Inflammation Research at the University of Edinburgh and Honorary Consultant Hepatologist at the Royal Infirmary of Edinburgh, looking after patients with a range of chronic liver conditions. During specialist training, he undertook a PhD in Southampton on the reversibility of liver fibrosis as an MRC Clinical Research Training Fellow. He relocated to Edinburgh in 2008 as an Academy of Medical Sciences/ Health Foundation Clinician Scientist Fellow. In 2014 he was awarded an NHS Research Scotland/ Universities Senior Clinical Fellowship. Professor Fallowfield's research interests span basic science and translational/ clinical studies in hepatology. Key topics include mechanisms of liver fibrogenesis and fibrosis regression; portal hypertension and hepatorenal syndrome; biomarkers (particularly imaging); and discovery/development of novel therapies for liver fibrosis, NASH and portal hypertension. Engagement with industry is a high priority theme.

Links:

https://www.ed.ac.uk/inflammation-research/people/principal-investigators/prof-jonathan-fallowfield

https://uk.linkedin.com/in/jonathan-fallowfield-80a7961

Prof. Fergal J. O'Brien, BA, BAI, PhD, FAS, CEng, FIEI, FEAMBES, MRIA

Director of Research & Innovation

Professor of Bioengineering & Regenerative Medicine

Head of Tissue Engineering Research Group

Dept. of Anatomy & Regenerative Medicine,
AMBER Centre,
Royal College of Surgeons in Ireland

GENE ACTIVATED SCAFFOLDS FOR ENHANCED TISSUE REPAIR

Fergal J. O'Brien 1,2

1 Tissue Engineering Research Group, Dept. of Anatomy & Regenerative Medicine &

2 Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland

Advances in tissue engineering have made progress towards the development of biomaterials with the capability for delivery of growth factors in order to promote enhanced tissue repair. However, controlling the release of these factors in order to maximise efficacy while limiting abhorrent side effects is a major challenge and has proved increasingly problematic in successful clinical translation. Gene therapy might be a valuable tool to avoid the limitations of local delivery of growth factors. While non-viral vectors are typically inefficient at transfecting cells, our group have had significant success in this area using a scaffold-mediated gene therapy approach for regenerative applications. These gene activated scaffold platforms not only act as a template for cell infiltration and tissue formation, but also as a 'factory' to provoke autologous host cells to take up specific genes and then engineer therapeutic proteins in a sustained but eventually transient fashion. In addition we have demonstrated how scaffold-mediated delivery of siRNAs and miRNAs can be used to silence specific genes associated with reduced repair or pathological states. This presentation will provide an overview of ongoing research in our lab in this area with a particular focus on gene-activated biomaterials for promoting bone, cartilage, nerve and wound repair. Focus will also be placed on advances we are making in using 3D printing of novel bioinks and electroactive biomaterials as next generation medical devices for tissue repair.

ACKNOWLEDGEMENTS: Funding from a European Research Council Advanced Grant, ReCaP (agreement n° 788753) .

Bio:

Fergal J. O'Brien is Director of Research & Innovation, Professor of Bioengineering & Regenerative Medicine, Head of the Tissue Engineering Research Group in RCSI and Deputy Director of the Advanced Materials & Bioengineering Research Centre (AMBER). He is a leading innovator in the development of advanced biomaterials for the repair of bone, cartilage, skin, respiratory, neural and other tissues. He has trained over 40 doctoral students to completion and published >250 articles in leading journals. His research has seen numerous patent filings, formation of an RCSI spin-out company and translation of technologies for bone and cartilage repair to the clinic. He is a recipient of numerous prestigious awards including a €3million Advanced Grant from the European Research Council (2018). He is a Fellow of Engineers Ireland (2013), the Anatomical Society (2014), and the European Alliance for Medical & Biological Engineering Science (2017) and is a Silver Medal recipient from the Royal Academy of Medicine in Ireland, and was elected to Membership of the Royal Irish Academy in 2018.

Brendan Harley, Sc.D.

Dept. of Chemical & Biomolecular Engineering

Cancer Center at Illinois

Carl R. Woese Institute for Genomic Biology

University of Illinois at Urbana-Champaign

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Building hierarchy: engineering porous scaffolds for regenerative medicine

Advances in the fields of tissue engineering and regenerative medicine require biomaterials that instruct, rather than simply permit, a desired cellular response. A major challenge to progress in our field is the complex organization of the tissues in our bodies, which are hierarchical, vary in space and time, and can differ person-to person. I will describe approaches to structurally and biomolecularly pattern biomaterials to enable tissue regeneration after injury. A major area of our work targets development of a degradable biomaterial to regenerate craniomaxillofacial bones and musculoskeletal insertions. I will describe the use of bioinspired design motifs and architected polymer foams to create composite materials that instruct desired cell activities while retaining mechanical competence required for clinical translation.

Bio:

Brendan Harley is the Robert W. Schaefer Professor in the Dept. of Chemical and Biomolecular Engineering at the University of Illinois at Urbana-Champaign. He received a B.S. in Engineering Sciences from Harvard University (2000), a Sc.D. in Mechanical Engineering from MIT (2006), and performed postdoctoral studies at the Joint Program for Transfusion Medicine at Children's Hospital Boston (2006 – 2008). His research group develops biomaterial platforms to dynamically regulate cell behavior for applications in musculoskeletal regeneration, hematopoietic stem cell biomanufacturing, as well as to investigate endometrial pathologies and invasive brain cancer. He has received funding from the NSF, NIH, American Cancer Society, the U.S. Army, and the AO Foundation. Prof. Harley co-founded a regenerative medicine company, Orthomimetics Ltd., to commercialize a biomaterial for osteochondral regeneration (global patent protection; CE Mark approval; 150+ patient Phase I clinical trial).

Dr. Harley has received a number of awards and honors including an NSF CAREER award (2013), the Young Investigator Award (2014) and the Clemson Award for Basic Research (2021) from the Society for Biomaterials (2014), as well as university research, teaching, and promotion awards (U. Illinois). He is an elected Fellow of the American Association for the Advancement of Science (2014) and the American Institute for Medical and Biological Engineering (2018).

Professor Molly M Stevens,
FREng, FRS

Professor of Biomedical Materials and Regenerative Medicine and

Research Director for Biomedical Material Sciences,

Department of Materials,

Institute of Biomedical Engineering,

Imperial College London

New materials based strategies for regenerative medicine

This talk will provide an overview of our recent developments in bio-instructive, self-assembling and gradient materials for regenerative medicine. We are developing biointerfaces with interesting features such as the incorporation of biological and topographical cues to enhance tissue regeneration and cell differentiation [1], and hydrogel-based materials with shape-memory and self-healing properties. We are using remote fields to engineer complex 3D architectures that mimic anisotropic and multiscale tissue structures and produce spatially arranged bioinstructive biochemical cues, such as acoustic stimulation to produce engineered muscle with bundles of aligned fibres [2]. I will present our advances in Raman spectroscopy characterisation techniques for high-throughput label-free characterization of single nanoparticles (SPARTA) [3]. Finally, I will discuss recent developments in our tunable nanoneedle arrays for multiplexed intracellular biosensing at sub-cellular resolution and modulation of biological processes [4]. These versatile technologies can be applied to a wide range of tissue engineering and therapeutic delivery applications.

Bio:

Prof Molly M Stevens FREng FRS is Professor of Biomedical Materials and Regenerative Medicine and the Research Director for Biomedical Material Sciences in the Department of Materials, in the Department of Bioengineering and the Institute of Biomedical Engineering at Imperial College London.

Prof Stevens' multidisciplinary research balances the investigation of fundamental science with the development of technology to address some of the major healthcare challenges. Her work has been instrumental in elucidating the bio-material interfaces. She has created a broad portfolio of designer biomaterials for applications in disease diagnostics and regenerative medicine. Her substantial body of work influences research groups around the world with over 30 major awards for the groups research and Clarivate Analytics Highly Cited Researcher in Cross-Field research.

Prof. Stevens holds numerous leadership positions including Director of the UK Regenerative Medicine Platform "Smart Acellular Materials" Hub, Deputy Director of the EPSRC IRC in Early-Warning Sensing Systems for Infectious Diseases and President of the Royal Society of Chemistry Division of Materials Chemistry. .

Dr Christine-Maria Horejs

Chief Editor, Nature Reviews Materials

Scientific writing and publishing – insights from a Nature editor

I this talk, I will provide an editorial perspective on publishing with Nature journals, discuss editorial processes, and provide tips and tricks for writing first-class research papers and Review articles.

Bio:

Christine received her MSc and PhD in nanobiotechnology from the University of Natural Resources and Life Sciences, Vienna, Austria, studying protein conformations and self-assembly using biophysical and theoretical approaches. She then joined the lab of Molly Stevens at Imperial College London, UK, investigating the extracellular matrix and cell–material interactions. In 2015, she moved to the Karolinska Institute, Sweden, conducting in vivo studies of anti-fibrotic biomaterials. In September 2017, she joined the Nature Reviews Materials team as an Associate Editor, and from July 2019 moved to be a Locum Senior Editor at Nature Nanotechnology, where she was primarily responsible for nanomedicine, drug delivery and nanobiotechnology. Since January 2021, she is Chief Editor of Nature Reviews Materials.

Abstract Presentations

Development of a triphasic 3D *in vitro* model for the regeneration of the bone-tendon-muscle interfaces

W. Balestri[1], R. H. Morris[2], J. A. Hunt [3,4], Y. Reinwald[1]

[1]Department of Engineering, Nottingham Trent University, Nottingham, GB [2]Department of Physics and Mathematics, Nottingham Trent University, Nottingham, GB [3] Medical Technologies and Advanced Materials, Nottingham Trent University, Nottingham, GB [4]College of Biomedical Engineering, China Medical University, Taichung 40402, Taiwan, TW

INTRODUCTION: Tissue interfaces are transition zones between individual tissues (Figure 1). Due to the interphases' limited ability to regenerate, injuries often result in prolonged pain, organ malfunction and an incomplete healing of injuries, followed by injury reoccurrence after surgery. While the biology and physiology of interfaces is well known, their development and regeneration is not fully understood.[1] This project aims to develop a 3D *in vitro* model to study the regeneration of bone-tendon-muscle interfaces. The behaviour and organisation of tissue-specific cell types when co-cultured on the 3D model will be analysed. The effects of surface topography and material composition in static and dynamic flow conditions will be evaluated.

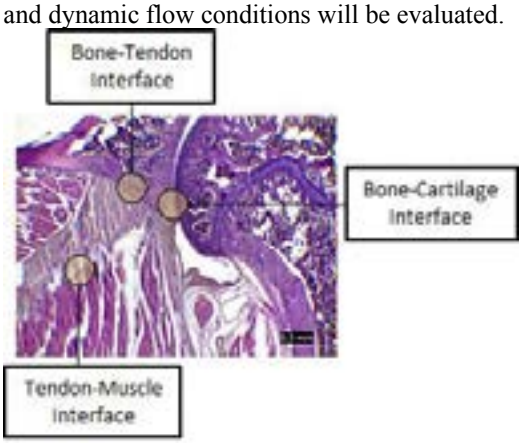

Figure 1: Orthopaedic interfaces. Histological stain of section of mouse joint. Image adapted from Balestri et al., 2020 [1]

METHODS: The 3D model was fabricated using inverted 3D printing. A stiffness gradient was developed by modifying ratios of type I collagen, agarose, and hydroxyapatite to resemble the native tissues. Moreover, physiological relevant surface topographies were designed. Then, MG63, human dermal fibroblast (HDF) and Sket.4U were characterized to establish their suitability as model for bone, tendon, and muscle, respectively. Cell proliferation, metabolic activity, morphology, and tissue-specific marker expression were evaluated. Optimal cell seeding densities allowing cells to reach homeostasis were determined. Cells were then co-cultured on the 3D model with and without stiffness gradient, with and without surface topographies.

RESULTS: Cells proliferated and were metabolically active over 14 days. Cells expressed tissue-specific markers. MG63 organised in aggregates and HDF and Sket.4U aligned in the same direction, but Sket.4 did not form fibres. A cell seeding densities of 50,000 cells/gel (MG63, HDF) and 100,000 cells/gel (Sket.4U) were chosen. Cells co-cultured on the 3D model without surface topography proliferated, were metabolically active and increased their total protein content over 14 days.

DISCUSSION & CONCLUSIONS: An in vitro model of bone-tendon-muscle was developed to study interface regeneration. An optimal cell seeding density was chosen for all cell type. Co-culture of tissue-specific cells showed their metabolic activity, proliferation, and protein production.

ACKNOWLEDGEMENTS: I would like to thank my supervisors for their support, the technical team of the Department of Engineering for the help, Gareth Williams for training on histology and Dr. Graham Hickman for the training with Atomic Force Microscope. Finally, thanks to Nottingham Trent University and MechAscan (EP/P031137/1) for funding.

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PCLMA & PGSM polyHIPE scaffolds for osteochondral regeneration

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INTRODUCTION: Osteochondral (OC) defects are one of the most common musculoskeletal (MSK) conditions in the UK. They are known for their low rate of healing and concomitant pathologies; they affect 3 out of 10 citizens over the age of 45. Every year, the NHS performs around 100,000 hip & knee replacements[1]. From these, over 90% of the patients are osteoarthritic. We propose an integrated biomaterial and tissue engineering (TE) approach as an early corrective solution.

METHODS: PCL and PGS prepolymer solutions were synthesised through ROP and condensation reactions. Further methacrylation was developed through the addition of methacrylic anhydride, using DCM as solvent. Prepolymer solutions were purified in an HCl aqueous solution. H+NMR and GPC were performed for chemical characterisation. PolyHIPE emulsions were manufactured photocuring under UV light. Moulding was used as AM process to creates mono disks. SEM imaging, water contact angle and mechanical testing were performed. Early cell work was done on both material disks, bulk and porous. BACs and hES-MPs were seeded on PGSM and PCLMA scaffolds respectively to assess cytotoxicity, cell attachment, proliferation, early migration, and ECM production. Viability and ECM production was performed through fluorescent imaging and histology assays.

RESULTS: High yield methacrylation was achieved by the control of number of free hydroxyl groups via methacrylic anhydride (MA) on PCL and PGS prepolymer solutions. Results developed photo and thermo-responsive polymers. DM was corroborated through NMR peaks on LM-PCL (35%) and HM-PCL (50%). PolyHIPEs were successfully manufactured through W/O emulsions. Emulsion stability was determined by experimenting with the following independent variables: solvent, speed & time of mixing, temperature of emulsion and volume of internal phase. Porosity and pore sizes have been tailored: smaller for chondral development and bigger voids for bone applications. Production of collagen and proteoglycans in chondral tissues, and collagen and calcium sulphates in bone, was observed to increase in reported pore sizes. Material shrinkage has been reported for PCL-HIPEs on 15-20%; for PGS around 30%, and stability over 25 days. Shrinkage, and degradation assays were run on PBS and alkaline environment. Phenotypes on the surface of scaffolds were identified after autoclaving, which can be attractive for cell attachment. Cell migration was measured with viability assays; an increase in proliferation reflected an increase in migration, confirmed by Alamar Blue residues. Scaffolds were imaged through SEM, confocal and LightSheet techniques; cellular-like bodies were identified. Qualitative measures for collagen, calcium sulphates, proteoglycans, and alkaline phosphatase were developed in 2D and 3D experiments on BACs, and hESMPs. Finally, the use of alginate hydrogels for cell encapsulation was successful for hES-MPs after 7 days with no reported natural polysaccharide degradation.

DISCUSSION & CONCLUSIONS: Both PCLMA and PGSM have mechanical properties suitable for OC applications. PolyHIPEs from both PCLMA and PGSM polymers possess a porous structure that allow cells and media to attach to their surface, to proliferate and to slowly migrate through the scaffold. PCLMA-LM and PGSM 80% have shown better results for early cell attachment and proliferation.

ACKNOWLEDGEMENTS: This research is possible through the support of my supervisors, my sponsor office CONACYT and The University of Sheffield.

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Development and characterisation of self-assembling peptide hydrogels as bioinks for 3D bioprinting applications

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[2]*School of Materials, Faculty of Science and Engineering, The University of Manchester*
[3]*Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester*

INTRODUCTION: With a growing demand for highly effective therapies, more sophisticated tissue-engineered models are required for a better understanding of the fundamental biological processes that underlie regeneration. To tackle this need and further comprehend these processes, 3D bioprinting has the scope to achieve well-defined biological structures by printing cell-embedded hydrogels or bioinks. One of the main challenges in obtaining fully functional constructs is the lack of optimal bioinks, which not only require good fabrication properties but also a cell-friendly extracellular matrix (ECM)-like microenvironment. Self-assembling peptide hydrogels (SAPHs) are fully defined and nature occurring hydrogels with tuneable mechanical properties. As a result, SAPHs stand as a powerful alternative to existing hydrogels in biofabrication and therefore, have the potential to fight one of the main limitations the 3D bioprinting field is facing at the time.

METHODS: Due to the lack of suitable biomaterials for 3D bioprinting applications, this research aims to design SAPHs as advanced bioinks for extrusion-based 3D bioprinting. For this purpose, rheological analyses and printability tests were carried out using oscillatory rheology and extrusion-based bioprinting.

RESULTS: Printability was assessed for the Manchester BIOGEL family of SAPHs, PeptiGel® hydrogels. Rheological analyses showed that peptide-based hydrogels are shear thinning and recover well under shear stress. Relaxation times fitting revealed the characteristic dynamic times in which our hydrogels recovered following the classical mechanical model. We further characterised the printability of the subject SAPHs through extrusion-based bioprinting. Here, printing resolution, shape fidelity and structural tests were undertaken. Printability experiments showed Alpha2 PeptiGel® is favourable in enhancing high-resolution constructs and therefore, comparable to materials with good fabrication properties. As to Alpha1 and Alpha4 PeptiGels®, despite they require further optimisation for biofabrication commitments, they presented beneficial bioink attributes. PeptiGels® were modified with sodium chloride tuning the ionic strength and therefore the hydrophobicity of the fibres within the hydrogel increasing its mechanical strength.

DISCUSSION & CONCLUSIONS: Thus far, we have successfully developed and tested peptide-based biomaterial inks. Current work aims to investigate their biocompatibility and potential as prospective cell-embedded bioinks for bone and cartilage fabrication.

The influence of fibrous scaffolds in electrical stimulation

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INTRODUCTION: Electrical Stimulation is an important tool for regenerating as well as engineering various functional tissues and is increasingly used in conjunction with conductive fibrous scaffolds. However, it is difficult to measure the direct input introduced to the cells. The aim of the study is to develop an in-silico model that estimates this input based on the electrodes potentials and is able to show the effects of different scaffold configurations on the cellular electric microenvironment.

METHODS: All operations, detailed in [1], were done using COMSOL Multiphysics 5.2® finite element analysis software. Five complexity stages of a fibrous scaffold model unit volume (Fig. 1) were created and parameterized. Laws of physics and boundary conditions were then added using the AC/DC module. Meshes were generated using the adaptive mesh refinement study. The set of governing algebraic equations was solved using the default solver configuration, and the numerical convergence criterion of 1e-3 estimated error was satisfied. The resulting electric field, current density and space charge density were then analysed.

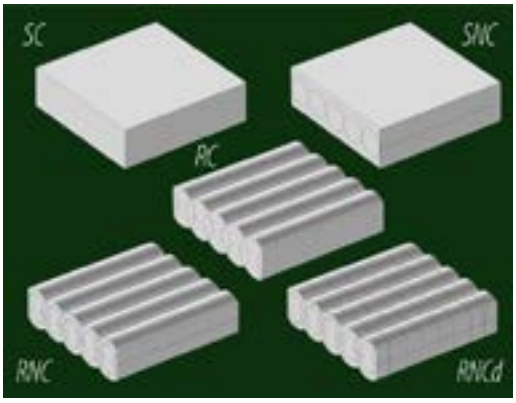

Fig. 1: Models of a fibrous scaffold: Smooth Conductive (SC), Smooth with Non-conductive Cores (SNC), Rough Conductive (RC), Rough with Non-conductive Cores (RNC, model of artificial fibers with conductive sheaths), Rough with Non-conductive Cores and alternate coats (RNCd, model of extracellular maxtrix collagen fibers banded structure).

RESULTS: Fig. 2 shows that model complexity and fibre orientation influence both the electric field intensity and charge around a fibrous scaffold. Importantly, the introduction of a fibrous scaffold increases the electric field intensity by up to 30%.

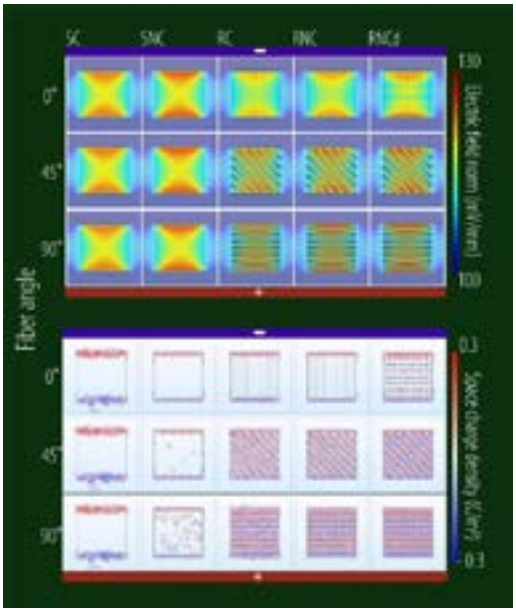

Fig. 2: Effects of model complexity and fiber orientation on the electric field intensity (top) and charge density (bottom).

DISCUSSION & CONCLUSIONS: This study shows that the scaffold configuration is important in the resulting cellular electric microenvironment, thus must be accounted for when designing electrical stimulation studies. Moreover, the contrast between the RNC and RNCd shows that artificial fibres are not yet a perfect replica of natural fibres.

ACKNOWLEDGEMENTS: This work was supported by the 4-year Wellcome Trust PhD Programme in Quantitative & Biophysical Biology

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Radiation Resistance Screening in a 3D Biomimetic Pancreatic Cancer Platform

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[2]Department of Physics, University of Surrey, Guildford, UK
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INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is a cancer of unmet clinical needs. Non-specific symptoms, high metastatic occurrence and treatment resistance elucidates to an extraordinarily low survival rates. PDAC encompasses a unique tumour microenvironment (TME). Hallmarks of the TME include dense desmoplasia and tumour hypoxia, are known to impair treatment responses. Tumour hypoxia has challenged radiotherapy for over 50 years. Radiotherapy for PDAC is traditionally controversial with contradictory clinical trial outcomes highlighting a need for further understanding optimisation of this treatment option.

Tissue engineering allows mimicry of bio-physical-chemical and mechanical properties of tumour microenvironments [5-7] to more readily recapitulate features of the tumour microenvironment that are responsible for treatment resistance. 3D models are emerging as useful tools for radiation screening for PDAC and are required for advancing radiation testing. *Utilising a 3D porous polymeric scaffolding system, here we aim to investigate hypoxia-induced radio-protection.*

METHODS: Fabrication of polymeric 3D scaffolds utilised the Thermally Induced Phase Separation method (TIPS) [1-3]. PANC-1 cells were seeded at $0.5x10^6$ and cultured for 4 weeks before being cultured in a hypoxic chamber (Baker InVivO2 300) (5% oxygen). Radiation exposures were performed using orthovoltage X-ray (250 kV). Samples were characterised at 24 hour, 3 days and 7 days post radiation. Confocal laser scanning microscopy enabled scaffold characterization of cellular organisation and viability and mapping of (radio-)protection via staining of live/dead, Caspase 3/7, HIF, actin, collagen and DAPI.

RESULTS: This research provides a platform for radiation responses in a 3D hypoxic PDAC system. Live/dead and Caspase 3/7 staining revealed hypoxic induced radioprotection.

DISCUSSION & CONCLUSIONS: These data show for the first time (i) a 3D polymeric scaffold supporting long-term hypoxic PDAC cell culture (ii) a long-term post-treatment *in situ* cell characterisation for radiotherapy treatment patterns. Live/dead and Caspase 3/7 staining suggested hypoxic induced radioprotection indicating a more realistic biomimetic research platform.

ACKNOWLEDGEMENTS: This research was supported by the Biological and Biochemical Engineering group (BioProChem), Department of Chemical and Process Engineering of the University of Surrey as well as the National Physical Laboratory, the EPSRC and the Royal Society. E.V. is grateful for a Royal Academy of Engineering Industrial Fellowship.

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DECELLULARISED PLEURAL MEMBRANE PATCHES FOR PROLONGED ALVEOLAR AIR LEAKS

Trisha Vikranth[1], Dr Tina Dale[1], Prof Nicholas R Forsyth[1]

[1]*School of Pharmacy and Bioengineering, Keele University, GB*

INTRODUCTION: Prolonged alveolar air leaks as post-surgical complications to routine lung resections and biopsies are a significant cause for patient morbidity. Transplantable decellularised pleural patches as adjuncts to conventional intraoperative closure techniques could reinforce the physical barrier, reducing incidence and severity of sustained air leaks. As a tissue engineered treatment modality, it can promote regeneration of the compromised pleura as a bioactive interface, stimulating endogenous repair mechanisms in the patient.

Adopting the classic paradigm of conditioning cells in a bio-instructive microenvironment niche, we focused on optimising protocols for isolation and expansion of porcine mesothelial cells (PMC) and porcine pleural membrane (PPM) decellularisation and characterisation.

METHODS: Cell culture studies looked at isolation efficiency of trypsin, pronase and direct explant culture for primary mesothelial cells from PPM, followed by their expansion and propagation in DMEM and cFAD medium to optimize culturing conditions favoring a mesothelial phenotype. Light microscopy, immunostaining and morphometric analysis were used to characterise established cell lines.

PPM decellularisation was carried out using physical (freeze-thaw cycles) and chemical (0.5% sodium deoxycholate and 1% Triton-X 100 in 10mMTris buffer) treatments. Decellularised PPM were characterised using histological and nuclear DNA staining, mechanical testing, and membrane thickness estimation.

RESULTS: Pronase digested cell cultures in cFAD medium, exhibited characteristic cobblestone morphology of a mesothelial-like phenotype, staining positive for mesothelin and vimentin. H&E staining of decellularised PPM showed absence of stained nuclei, consistent with significant reduction ($p<0.0001$) of DAPI stained nuclei counts against native controls.

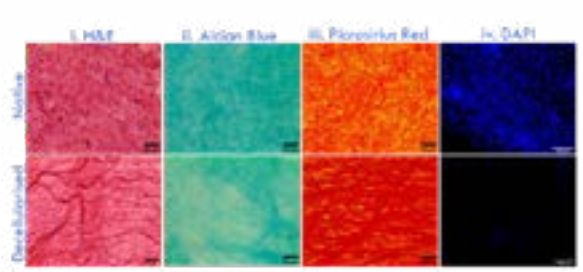

Figure 1 Histological analysis of Decellularised PPM. (i) H&E (ii) Alcian blue for GAGs (iii) Picrosirius red for collagen.

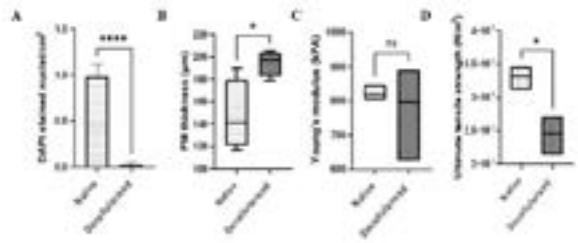

Figure 2 Quantitative assessments of the effect of decellularisation on (A) Nuclear membrane integrity (**p < 0.001). (B) Membrane thickness (*p< 0.05). (C) Mechanical integrity (P>0.05). (D) Ultimate tensile strength (*p<0.05)**

Alcian blue staining for glycosaminoglycans (GAG) and Picrosirius red staining for collagen exhibited comparable staining profiles and intensities in decellularised PPM, suggestive of minimal disruption to structural alignment and composition of the native ECM. Decellularised PPM were estimated to be thicker (218 ± 67µm) than the native controls (145 ± 33µm). The derived Youngs modulus for the treated membranes (804.08 ± 670.49 kPa) through uni-axial tensile testing, were comparable to the native controls (828.44 ± 177 kPa).

CONCLUSION: Our pilot study represents a step forward in deriving bioactive ECM scaffolds in the form of decellularised PPM. Next step is in expanding the characterisation regime to include proteomics and ultrastructural studies. Assessing recellularisation potential of the derived scaffolds using the established mesothelial cell lines, will underpin our research focus towards developing proof of concept for a biological scaffold-based therapeutic approach.

Using nitric oxide as a reliable marker to detect macrophages' activation state

I.Kalokoh[1], Y.Yang[1]

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INTRODUCTION: Macrophages in innate immune system become activated when recognizing the molecular patterns associated with pathogens or internalization of foreign materials. It has been reported that nitric oxide (NO) is a effector molecule released by macrophages as a toxic defense substance against infectious organisms. In this study, we would like to study whether we can use nitric oxide as a reliable marker to detect macrophages' activation state and associated factors.

METHODS: Human monocytes cell line, THP-1, was used to establish model innate immunesystem. THP-1 cells were activated to macrophage or M0 phenotype using PMA first, and then the cells were induced to M1 macrophages using two inducers, LPS and IFN-r. The cell seeding density were varied, 10,000, 20,000 and 50,000 cells per well in 96 well plate to assess the effect of cell number on NO production concentration. All cells were cultured up to 6 days. The cell viability and NO production were measured by CCK8 kit and Griess assay at day 2, 4, 6 respectively.

RESULTS: Visible imaging revealed that macrophage at M0 state exhibiting more round morphology, whilst M1 phenotype cells showing more elongated morphology (Figure 1).

Fig. 1: M0 morphology (top) vs. induced M1 (bottom). Magnification X20.

On day 2, cells polarity increased for all the different cell number groups and decreases on day 4 and 6 due to cell apoptosis. For the NO assay, there were more NO release on day 2 of all different cell numbers as there were more live cells on day 2 than the other days. Inactive macrophages M0 produced far low NO than active state, M1. LPS+IFN- γ activated M0 cell effectively (Figure 2)

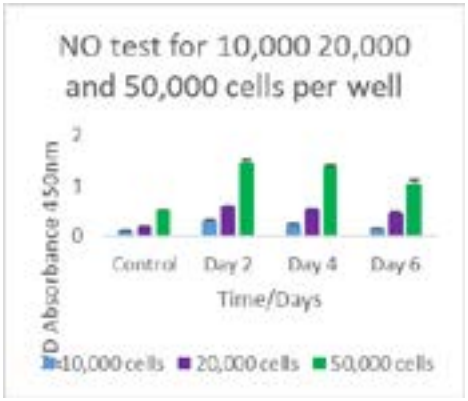

Figure 2: NO release for M1 cells at different cell density groups of 10,000 20,000 and 50,000 cells per well at culture time 2, 4, 6 day in comparison to that of M0 (10,000 cells per well).

DISCUSSION AND CONCLUSION: High cell number of M1 produced higher concentration of NO. Longer culture time reduced M1 cell number and reduced NO production. Cell viability tests correlated NO assay. Greiss buffer assay is a simple and reliable test for macrophage activation.

ACKNOWLEDGEMENTS: This project is partially support by EPSRC CDT regenerate medicine programme.

REFERENCE: [1]Kalokoh I S et al Recent Progress in Immune cells and Biomaterials.

Ultrasound-responsive nanodroplet formulations for triggered release of a GSK3 antagonist for bone fracture healing

Jonathan P. May,[1,2,4] Anastasia Polydorou,[1,2] Sara Ferri,[1,2,4] Qiang Wu,[3] Eleanor Stride,[3] Dario Carugo,[1,4] Nicholas D. Evans [1,2]

[1]Bioengineering Science Research Group, Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, UK; [2]Bone and Joint Research Group, Human Development and Health, Faculty of Medicine, Southampton General Hospital, Southampton, UK; [3]BUBBL Group, Institute of Biomedical Engineering, University of Oxford, Oxford, UK; [4]School of Pharmacy, University College London (UCL), London, UK

INTRODUCTION: Impaired fracture healing impacts patients' quality of life and imposes a financial burden on healthcare services. Up to 10% of bone fractures result in delayed or non-union fractures, for which new treatments are urgently required [1]. However, systemic delivery of bone anabolic molecules is often sub-optimal and can lead to significant side effects. In this study, we developed ultrasound (US) responsive nano-sized vehicles in the form of perfluorocarbon nanodroplets (NDs), as a means of targeting delivery of drugs to localised tissues [2]. We tested the hypothesis that NDs could stably encapsulate BIO (GSK-3β inhibitor), which could be released upon US stimulation to activate Wnt signaling and induce ossification [3].

METHODS: NDs (~280nm in diameter) were prepared from phospholipids and liquid perfluorocarbon and their stability and drug loading was studied by nanoparticle tracking analysis (NTA) and HPLC. ND cytotoxicity was assessed in patient derived, bone marrow stromal cells (BMSCs) with Alamar Blue (24h), and in vitro bioactivity of BIO-NDs was evaluated in a 3T3 Wnt-pathway reporter cell line with luciferase readout. To investigate the acoustic behaviour of NDs, 2% agarose (LM) containing NDs was injected into a bespoke bone fracture model (Sawbones) of various geometries and stimulated by US (1 MHz, 5% duty cycle, 1MPa, 30s), allowing simultaneous capture of optical images and acoustic emissions. Femoral bone hole defects (1-2mm) were made in WT-MF1 mice (age: 8-12wks) and DiR-labelled NDs (100µL, 10^9NDs/mL, i.v.) were injected post-fracture to determine biodistribution by IVIS imaging.

RESULTS: NDs were stable (at 4 and 37°C) and retained > 90% BIO until US was applied, which caused ~100% release. ND exposure up to a concentration of 10^9 NDs/mL showed no cytotoxicity (24h). BIO-loaded NDs induced Wnt pathway activation in a dose dependent manner. Biodistribution of DiR-NDs in a femoral bone hole defect model in mice demonstrated increased localisation at the fracture site (~2-fold relative to that found in healthy mice or contralateral femurs at 48h). In the bespoke bone fracture model, acoustic emissions indicative of ND vaporisation were detected when a driving pressure of 0.5 MPa was exceeded at 1 MHz.

DISCUSSION & CONCLUSIONS: The present study supports the hypothesis that NDs may provide a means of US-induced localised release of BIO at bone fracture sites to promote Wnt signalling. Evidence that NDs passively localise at bone fracture sites provides encouragement that minimally invasive and localised delivery of anabolic agents may be possible using US-responsive nano-sized delivery agents.

ACKNOWLEDGEMENTS: We acknowledge the support of EPSRC for funding and the expertise of the Biomedical Research Facility staff at the University of Southampton.

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Identification and isolation of progenitor cell subpopulations within the adult nucleus pulposus: implications for intervertebral disc regeneration

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INTRODUCTION: The central nucleus pulposus (NP) of the developing human intervertebral disc (IVD) is occupied by large, vacuolated notochordal cells (NCs) which are lost after the first decade of life. Loss of NCs typically coincides with the onset of IVD degeneration and associated back pain, therefore these cells are hypothesised to be important in NP homeostasis. We[1], and others[2], have previously demonstrated that the adult human NP holds NC-like/progenitor cell subpopulations. Here, we aimed to apply a panel of markers proposed to identify NC-like (CD24) and NP progenitor cell (TIE2 and GD2) populations and isolate them using flow cytometry in order to assess their phenotype and regenerative potential.

METHODS: The presence of CD24+ cells within the adult NP was confirmed through immunohistochemistry across age and degeneration. Flow cytometry was then used to analyse CD24 expression in a cohort of adult human NP samples across a wide age and degeneration grade range (20-76 years, 3-12 degeneration grade, n=9) and to determine whether expression changed with time in culture. Moreover, FACS sorting for CD24 was used to isolate NC-like (CD24+) and CD24- cells from cryopreserved human adult NP cells (27-57 years old, n=3), which were then returned to culture. Subsequently, flow cytometry and kinetics analysis was performed at consistent intervals to monitor the marker expression changes of CD24 and the NP progenitor markers (Tie&GD2) with time in culture.

RESULTS: Immunohistochemical staining of paediatric and adult IVD tissue localised sub-populations of NP cells positive for CD24 through ageing and degeneration. All nine adult human samples analysed through flow cytometry showed varied starting CD24 positivity ranging from 1.93% to 29.4%; positivity which increased across all samples with time in culture and plateaued after two passages, irrespective of age and degeneration grade. When looking at the expression changes of the selected markers (CD24, TIE2 and GD2) in the sorted CD24 + & - populations through time in culture, a double positive CD24 and GD2 subpopulation was identified in the positive population, which was observed across all passages. CD24 expression decreased with time in culture and no Tie2 positive cells were observed in either population. Population doubling times (PDT=8 days for CD24+ and 8 ½ days for CD24- cells) and growth rates (r= 0.087 for CD24+ and 0.080 for CD24- cells) did not significantly differ between the CD24+ and – populations.

DISCUSSION & CONCLUSIONS: Together, these results highlight the presence of an NC-like CD24+ population within the adult human IVD irrespective of age or degeneration. The fluctuations in CD24 expression observed, as well as the population kinetics results, suggest that culturing conditions might influence CD24 expression when kept in culture. The finding of a subpopulation double positive for CD24 and GD2 in the adult human NP opens up the possibility of a novel progenitor-like subpopulation. This suggests that cell surface marker GD2, if paneled up with the routinely used CD24 marker, could lead to an improved isolation of notochordal-like cells from the adult NP. Work is ongoing to establish the phenotype and regenerative potential of the identified sub-populations for use in the development of new strategies for treatment of IVD degeneration and associated back pain.

ACKNOLEDGEMETS: I would like to thank my funding body, BBSRC.

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Biosafety of graphene oxide in an in-vivo murine model

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INTRODUCTION Graphene is a highly conductive and adhesive biomaterial that can be modified due to its physicochemical properties. The salient features of graphene can be seen through reports of antibacterial [1] and anticancer activities [2], making it a promising candidate for several biomedical applications, including tissue engineering scaffolds [3]. However, contradictions in literature due to potential toxicity from residual impurities and inconsistent therapeutic dosage reports make it a challenge to translate its application into humans, hence, we investigate here the biocompatibility of graphene oxide (GO).

METHODS This study compared commercially obtained graphene oxide (GO CBG) and graphene oxide synthesized in the lab (GO P1_2) in an *in vivo* murine model using histopathological examinations. The work was approved by the bioethical commission, protocol №63, 09.10.2020, Novosibirsk Institute of Cytology and Genetics. Briefly, adult male rats (n=70) were intravenously injected with sterile water (control group; n= 10), graphite(n= 20), GO-CBG(n=20), or GO-P1_2(n=20) at a dose range of 0.5mg/kg to 75mg/kg for 2 weeks. The organs, including heart, kidney, liver, spleen and lungs, were retrieved for morphological analysis along with brain tissue to determine any blood-brain barrier (BBB) penetration.

RESULTS No mortality or signs of overdosing were observed in any group. Comparative analysis of mice that received 50mg/kg and 75mg/kg of GO revealed no significant changes in any organ tissues except in the lung tissue. Slight changes in the morphology in the GO P1_2 group and large conglomerates in the GO CGB group were observed (Figure 1). The quantity of GO varied across the tissues: GO P1_2 group showed larger quantities of GO compared to GO CBG group, except in the heart tissue where no difference was seen. GO P1_2 was concentrated in the liver (cytoplasm of Kupffer cells), spleen and lungs (walls of alveolar sacs), while the heart and kidney tissues had minimal traces of either substance. No traces were seen in the brain tissue suggesting no penetration of BBB.

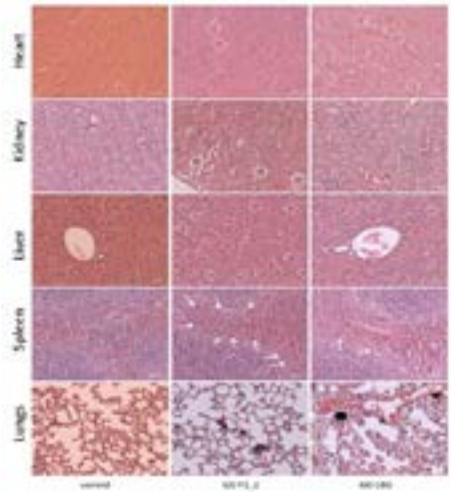

Figure 1 Tissues of a mouse that received an intravenous injection of GO at the highest dosage of 75mg/kg. White circles, white arrows and red arrows show GO particles. Image is obtained at x20 magnification.

DISCUSSION & CONCLUSION The study did not show any apparent difference in this dosage range. Accumulation of GO in most organs coincides with the localization of macrophages which can be helpful in future application. The changes in the morphology of lung tissues can neither be pathological or normal, but large conglomerates in the pulmonary vessel may lead to thrombosis and impair lung function. Additional research on an acute toxicity study in higher doses of GO is required.

Acknowledgement
This work was supported by the British Council Workshop grant 2018-RLWK10-458243278.

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CHEMOTHERAPEUTIC ASSESSMENT ON A NOVEL SCAFFOLD ASSISTED MULTICELLULAR MODEL OF PANCREATIC CANCER

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INTRODUCTION: The aggressive nature and high mortality rate of PDAC are attributed to its late diagnosis, heterogenous nature and its resistance to currently available treatment methods. 3D *in vitro* models are considered to have better *in vivo* niche mimicking capabilities in comparison to 2D culture systems while mitigating the cost and reproducibility problems associated with animal models. Additionally, as in all tissues, the PDAC tumour microenvironment is heterogeneous in cellular nature consisting, additionally to cancer cells, of different cell types, e.g., stellate cells and endothelial cells, all contributing to the tumour formation, metastasis as well as its response and resistance to treatment. Thus, recent studies have focused on generating multicellular pancreatic cancer models, which are primarily spheroid based[1]. Based on our previous work on polymeric assisted 3D PDAC monocellular models[2,3], we have recently developed a 3D hybrid multicellular model of pancreatic cancer using pancreatic cancer cells, endothelial cells and pancreatic stellate cells, wherein we were able to show long term viability, feasibility of extracellular matrix (ECM) mimicry through both scaffold coating and substantial matrix secretion from the stellate cells (desmoplasia recapitulation), formation of dense cellular masses and migration between the stroma and tumour[4]. *The current work focusses on the feasibility of using this multicellular model for the purposes of chemotherapy screening. We also look at the effects of a zonal vs a single scaffold based multicellular model* on the treatment response.

METHODS: PU scaffolds were prepared using Thermal Induced Phase Separation (TIPS) method. Absorption based surface modification of the scaffolds enabled coating with ECM proteins for enhancement of ECM mimicry. A zonal structure with (i) endothelial and stellate cells on the outer side of the scaffold coated with collagen I and (ii) pancreatic cancer cells in the inner scaffold coated with fibronectin was designed, along with a single scaffold based simplistic multicellular model[4]. 50µM Gemcitabine was applied to the model after 4 weeks of culture, followed by 7 days observation post treatment. *In situ* assays for monitoring cell viability, spatial organisation, ECM production were carried out at specific time points throughout the culture period.

RESULTS: We report chemotherapeutic assessment on our novel multicellular model of PDAC. Effects of therapeutic agents on cell viability, apoptosis ECM secretion for both the single scaffold and complex zonal model are observed.

DISCUSSION & CONCLUSIONS: Our data show, that our multicellular model is an appropriate *in vitro* model for therapeutic assessment of PDAC. Our developed model is a low-cost high throughput tool that can be used for personalized studies and treatment screening of pancreatic cancer.

ACKNOWLEDGEMENTS: Financial support was received from the Department of Chemical and Process Engineering, an Impact Acceleration Grant (IAA-KN9149C) from the University of Surrey, an IAA–EPSRC Grant (RN0281J) and the Royal Society. P.G has received financial support from Commonwealth Rutherford Post-Doctoral Fellowship (2018–2020) and the 3D BioNet (UKRI). E.V. is thankful to the Royal Academy of Engineering for an Industrial Fellowship.

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Hydroxyapatite-decorated Fmoc-hydrogel as a bone mimicking substrate for osteoclast differentiation and culture

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INTRODUCTION: Hydrogels are biomaterials that recapitulate many of the extracellular matrix properties in tissue engineering applications [1]. However, their use in bone regeneration is limited due to a lack of mineralization and poor mechanical properties [2]. We present here new data on a composite hydrogel based on the self-assembling gelator peptides Fmoc diphenylalanine/serine (Fmoc-FF/S) and Fmoc-RGD, which has been modified by incorporation of hydroxyapatite (Hap) nanopowder as "nanofiller". These new hydrogels systems could be used as proof of principle to build a more complex bone-mimicking substrate for culturing osteoclasts than typical tissue culture methods and offer a more reliable platform for bone regeneration studies.

METHODS: Biogelx hydrogels were prepared following the manufacturer protocol. Hap nanopowder was incorporated into the hydrogel at different ratios (w/v). Mechanical properties and topography of the resulting hydrogels were analysed via Rheometry and AFM. Raw 264.7 cells were used as a pre-osteoclast model to assess the response of a bone-like cell line to the Hap-decorated hydrogels. Cell morphology was evaluated using F-actin staining. Formation of mature osteoclasts was assessed morphologically by F-actin staining and by TRAP immunofluofluorescence staining. Expression of typical osteoclast markers was also evaluated by gene expression.

RESULTS: Hap-decorated hydrogels showed a higher storage modulus and improved mechanical properties than the Hap-free hydrogels. Moreover, the hydroxyapatite can "decorate" the Fmoc-RGD hydrogels without impairing the hydrogels self-assembling mechanism. Additionally, the hydrogels supported cell adhesion and cell viability in vitro. Interestingly, Hap-decorated RGD-hydrogels were able to support osteoclastogenesis in vitro as Raw 264.7 cells showed typical morphology of mature osteoclasts without prior addition of RANKL. Moreover, the expression of typical osteoclast markers and the presence of TRAP-positive cells suggests that Raw 264.7 cells had differentiated to mature osteoclasts.

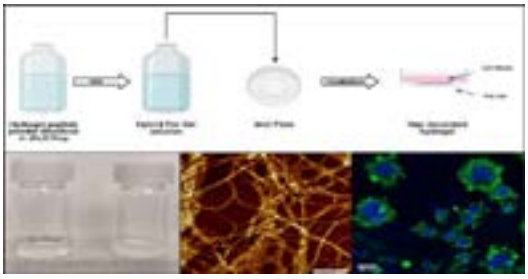

Figure 1*. Overview of the developed Hap-decorated hydrogels (upper panel). Lower panel shows AFM topography of the Hap-decorated hydrogels fibres and F-actin staining of the Raw264.7-derived osteoclast.*

DISCUSSION & CONCLUSIONS:
We have developed a new hydroxyapatite-decorated RGD-peptide hydrogels that shows enhanced mechanical properties and that is able to trigger the Raw 264/7 differentiation toward mature osteoclast, despite the lack of stimulating growth factor. These results suggest thatthis new biomaterial could be used as a template for studying the differentiation and culture of osteoclasts and ultimately could offer improved bone regeneration substrates.

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Epigenetic reprogramming promotes the therapeutic potency of osteoblast-derived extracellular vesicles for bone regeneration

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INTRODUCTION: For bone regeneration, there is great precedence to develop instructive acellular technologies that circumvent limitations associated with the translation of cell-based therapies. Extracellular vesicles (EVs) derived from osteoblasts enhanced stem cell mineralisation compared to BMP-2. Regulating the cell's epigenetics through histone deacetylase (HDAC) inhibition enhances their differentiation potential. Therefore, this study investigated altering osteoblasts epigenetic function via the HDAC inhibitor Trichostatin A (TSA) to promote osteoblast-derived EVs potency.

METHODS: TSA effect on osteoblast epigenetic functionality and mineralisation was determined by quantifying HDAC activity and calcium deposition. EVs were isolated from untreated/TSA treated osteoblasts for 2 weeks. EV size and concentration were defined using nanoparticle tracking analysis. EVs microRNA and protein expression was evaluated using microarray and proteomics analysis. Osteogenic differentiation of human bone marrow stromal cells (hBMSCs) cultured with untreated (MO-EVs)/TSA treated osteoblast-derived EVs (TSA-EVs) was evaluated by qPCR, biochemistry and histological analysis.

RESULTS: TSA significantly reduced osteoblast HDAC activity and enhanced histone acetylation and calcium deposition when compared to untreated cells in a dose-dependent manner (Fig 1). The quantity of EVs generated, in addition to their protein content and size correlated with the degree of osteoblast differentiation. MicroRNA profiling revealed TSA-EVs were enriched with several pro-osteogenic microRNAs were involved in regulating pathways such as the Wnt signalling pathway. Moreover, proteomics analysis identified the enrichment of proteins involved in transcriptional regulation within TSA-EVs.

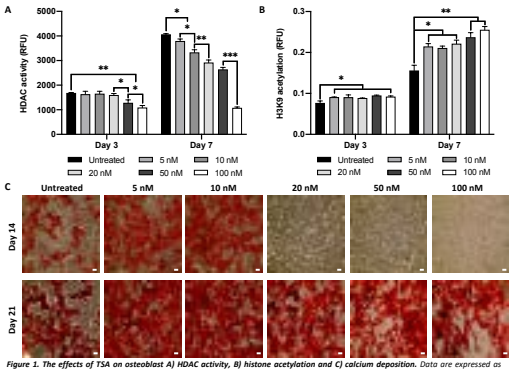

Figure 1. The effects of TSA on osteoblast A) HDAC activity, B) histone acetylation and C) calcium deposition. *Data are expressed as mean ± SD (n=3). *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001.*

Importantly, TSA-EV treatment significantly upregulated hBMSCs osteoblast-related gene/protein expression (ALP, Col1a, BSP1, OCN) and promoted extracellular matrix mineralisation when compared to MO-EVs treatment during osteogenic culture (Fig 2).

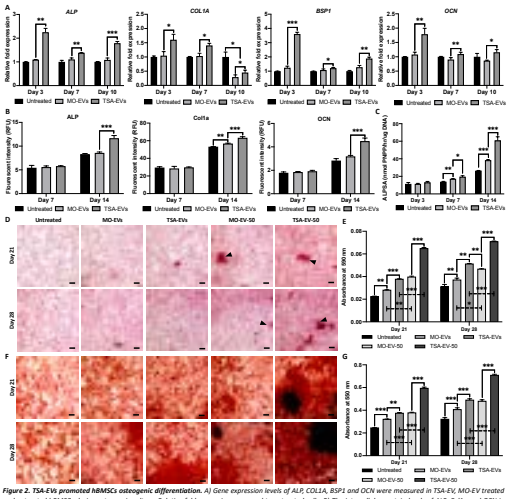

Figure 2. TSA-EVs promoted hBMSCs osteogenic differentiation. *A) Gene expression levels of ALP, COL1A, BSP1 and OCN were measured in TSA-EV, MO-EV treated and untreated hBMSCs during osteogenic culture. Relative fold expression compared to untreated cells. B) The intracellular protein levels of ALP, Col1a and OCN in EV treated hBMSCs analysed by ICW. C) The effects of TSA-EVs on hBMSCs ALP activity. D) Picrosirius red staining for collagen production of EV-treated hBMSCs. Black arrows highlight mineral nodule-like structures. E) Quantitative analysis of collagen staining. F) Alizarin red staining for calcium deposition on EV-treated hBMSCs. Black staining indicates mineral formation. G) Qualitative analysis of alizarin red staining. Scale bars = 200 µm. (MO-EV, TSA EV, 10 µg/ml) (MO-EV-50, TSA-EV-50, 50 µg/ml). Data are expressed as mean ± SD (n=3). *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001.*

DISCUSSION & CONCLUSIONS: Together, epigenetic reprogramming provides a novel engineering approach to enhance the therapeutic efficacy of osteoblast-derived EVs as an acellular tool for bone augmentation strategies.

OPTOGENETIC CONTROL OF BMP SIGNALLING TO DRIVE HPSC CHONDROGENESIS

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INTRODUCTION: The Transforming Growth Factor β (TGFβ) superfamily (including Bone Morphogenetic Proteins (BMPs)) are a family of ligands crucial in chondrogenic development. TGFβ molecules transduce signals through Type I and II receptors that phosphorylate members of the SMAD family in canonical signalling. These then translocate to and accumulate within the nucleus to initiate transcription. Chondrogenic differentiation of human pluripotent stem cells (hPSCs) relies upon supplementation of recombinant growth factors to active TGFβ/BMP signals, but this may lead to poor differentiation reproducibility and quality because of batch-to-batch variation and growth factor degradation. An alternative and more precise means of receptor activation may enable refinement of differentiation. A novel approach is to utilise optogenetics: the control of cells with light. Optogenetics offers advantages over traditional means, as it provides an on/off reversible signalling system that can be finely tuned through modulation of light wavelength, intensity and frequency.

METHODS: Optogenetic BMP-like receptors were generated through NeB Hifi Assembly; consisting of the intracellular regions of BMPR1B or BMPR2 chimerised to a myristoylation signal at the N-terminus for membrane anchorage and the light sensitive Light Oxygen Voltage (LOV) domain at the C-terminus for light-induced dimerisation. Expression of optogenetic constructs was controlled by addition of doxycycline. Optogenetic activation of canonical BMP signalling was analysed through detection of nuclear P-SMAD1/5, activation of a SMAD1/5/8 transcriptional reporter (BRE) and upregulation of relevant BMP-target gene expression. MAN13 hPSCs were differentiated towards chondrocytes using an established protocol (1). BMP-like growth factors were substituted for an optimized regime of light stimulation.

RESULTS: Optogenetic receptor transgenes were successfully generated and integrated into HEK293T, TC28a2 and MAN13 hPSCs Expression of the optoBMP system was regulated by the addition of doxycycline. Blue light illumination of optogenetic cell lines induced phosphorylation and nuclear accumulation of SMAD1/5, indicating optogenetic activation of the canonical BMP pathway. SMAD1/5/8 transcriptional activity was activated by light stimulation, demonstrated through BRE activation and upregulation of BMP-like gene expression (2). Optogenetic stimulation during hPSC chondrogenic differentiation indicated optoBMP activation could substitute for BMP growth factors and drive differentiation.

DISCUSSION & CONCLUSIONS: Optogenetic stimulation during hPSC chondrogenic differentiation indicated optoBMP activation could substitute for BMP growth factors and drive differentiation. Furthermore, analyses indicated that optogenetic stimulation initiated a divergent signalling response to that of BMP2 growth factor stimulation, which then facilitated significantly higher upregulation of key chondrogenic genes, including *COL2A1* and *SOX9*. Findings documented here demonstrate that optogenetics can be utilised to control the BMP signalling pathway and has illustrated the potential for manipulation of chondrogenic processes during hPSC differentiation.

ACKNOWLEDGEMENTS: The authors acknowledge the support of the EPSRC and MRC CDT in Regenerative Medicine.

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Bone and Tissue Engineering of the Temporomandibular Joint (condyle): A Histological Investigation of the Rat Condyle, Knee and Hip.

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INTRODUCTION: The temporomandibular joint (TMJ) is a complex osteochondral joint which is made up of the articular disc and condyle (1). The histological structure of the rat condyle, knee and hip was investigated to determine whether there are any similarities or differences between the different types of osteochondral joints and whether orthopedic tissue engineering approaches can be used to tissue engineer the condyle. It will also determine whether the rat is a suitable animal testing model. 4 rats' condyles, knees and hips were examined in this study.

METHODS: Two rats were dissected using the Fuentes *et al.*, (2017) dissection protocol. The rat tissue underwent the correct processes to be fixed and stained (2). Haematoxylin and eosin (H&E), picrosirius red (PSR) and alcian blue Scotts Method (AB/SM) stains were used following the University of Manchester staining protocols.

RESULTS:

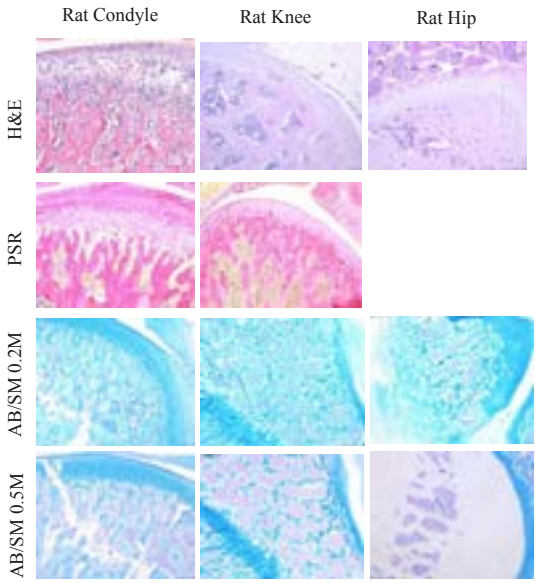

Fig. 1: H&E stain (top) PSR stain (middle) AB SM 0.2M (second from the bottom) AB SM 0.5M (bottom). Rat condyle (left) rat knee (middle) rat hip (bottom).

DISCUSSION & CONCLUSIONS: The H&E stain identified the similarities in the rat condyle, knee and hip. The similarities lay between the cellular structure and four layers of the cartilage to bone. The following were found amongst the layers; the fibrous layer highlighted dense fibrous connective tissue with scattered flat cells. The proliferative layer showed irregular, densely packed cells with large round nuclei. The chondrocytic layer found spherical cells with an increase of depth, and the hypertrophic layer highlighted a higher presence of chondrocytes. The PSR stain highlighted the presence of collagen with no presence of collagen type I or III as reported in previous literature in human condyles (2). The rat knee showed a higher presence of collagen bundles in comparison to the rat condyle. The AB/SM stain revealed sulphated acid mucopolysaccharides were prominent at 0.2M throughout all four layers of the rat condyle, knee and hip. 0.5M highlighted the presence of strongly sulphated acid mucopolysaccharides scarcely present throughout all four layers in the rat condyle, knee and hip. From the histological investigations that were undertaken, it can be concluded that many similarities have been found between the rat condyle, knee and hip. However, further research needs to be undertaken to better understand the anatomical structure of the human condyle, in particular the differences between the types of cartilage lining the joints, before drawing a wider conclusion on whether orthopedic tissue engineering approaches can be used to tissue engineer the condyle. whether a rat is a suitable animal model for *in-vitro* testing.

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Identifying mechanical loading parameters to study the mechanobiology of herniation using a computational model

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INTRODUCTION: The incidence of abdominal hernias increases with intrinsic weakness of the abdominal wall or increased intra-abdominal pressure (IAP) (1). The latter involves abnormal loading, such as that seen in chronic coughing. This is especially seen in the rectus sheath, a fibrous connective tissue layer involved in abdominal herniation (2). Current treatment involves surgical repair, whereby the protruding tissue is pushed back into the abdominal cavity and a mesh is implanted for mechanical support. Poor prognosis post mesh implantation may be attributed to cellular and mechanical aberrations that are not corrected within treatment.
The aim of this study is to investigate how external loading impacts the local mechanical properties of the rectus sheath, and how that impacts on cell behaviour.

METHODS: Loading of the rectus sheath is investigated through a computational modelling approach utilising finite element analysis on FEBio Studio 1.4.2. A transversely isotropic Mooney-Rivlin model for an incompressible, hyperelastic material is applied to a (60 × 315 × 0.655 mm) model of the rectus sheath. The tissue is subsequently loaded with a surface pressure onto one z face whilst both y faces are fixed, mimicking the physiological conditions of the tissue. Different loads are applied corresponding to the following activities: resting, 0.67 KPa; resting pregnancy, 1.87 KPa; resting incisional hernia, 2 KPa; resting obesity, 2 KPa; resting abdominal compartment syndrome (ACS), 2.67 KPa; standing, 2.67 KPa; standing cough, 10.85 KPa; jumping, 23.33 KPa.

RESULTS: In all loading conditions, the tissue experiences the largest displacement at the centre (Figure 1) and the largest stress is found where the tissue is fixed. This pattern becomes more apparent as the magnitude of the incident load increases. A large increase in displacement and stress is experienced from standing to standing cough, corresponding with the large increase in pressure. A stress-strain plot is generated with FEBio and the Young's Modulus is found to be 0.0406 MPa.

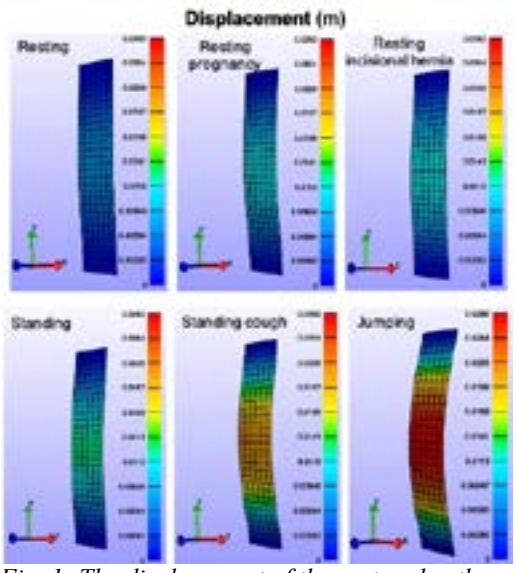

Fig. 1: The displacement of the rectus sheath under different activities.

DISCUSSION & CONCLUSIONS: Through this model, the stress, strain, displacement and pressure of the rectus sheath is characterised, enabling an understanding of the loading parameters involved in herniation compared to physiologic activities. This will provide the fundamental parameters required to test 3D tissue models and animal samples of the rectus sheath, with an intent to study the impact of these forces on the cells of the abdominal wall.
Further statistical analysis is required to determine the significance of these findings. Future work includes the addition of other abdominal tissue layers to improve the complexity of the model.

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Bio-instructive Collagen-Magnesium Nanocomposite Scaffolds to Manipulate Bone Metabolism and Promote Bone Repair

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INTRODUCTION: Bone remodelling and regeneration involves the balance of bone resorption (catabolism), and bone formation (anabolism)[1]. Imbalances in bone remodelling represent an underlying cause of metabolic bone diseases such as osteoporosis, and present a major risk for implant loosening. Herein, we focus on investigating a combined pro-anabolic and anti-catabolic effect of Magnesium (Mg^{2+}) bioactive ions[2] to develop a biomimetic bio-instructive biomaterial scaffold structurally designed to enhance bone formation while impeding uncontrolled osteoclast resorption activities to facilitate better bone regeneration and promote repair.

METHODS: *In vitro* 2D model: Pre-osteoblasts MC3T3-E1 and osteoclast progenitors RAW 264.7 were cultured in osteogenic medium and 20 ng/ml RANKL stimulated medium, respectively and, exposed to increasing concentrations of $MgCl_2$ ranging from 0-25mM, to determine the effects of extracellular Mg^{2+} on the proliferation, mineralisation, TRAP activity, and gene expression of bone cells. *In vitro* 3D model: Nano-hydroxyapatite nanoparticles (nHAp) doped with 10mM (10Mg nHAp) and 25mM (25Mg nHAp) of $MgCl_2$ were incorporated into freeze-dried type I collagen sponge-scaffolds, and physicochemically characterised via FTIR, SEM, water contact angle, and mechanical testing. Scaffolds were cultured separately with MC3T3-E1 and RAW 264.7 cells, and assessed for cell viability using Live/Dead assay, proliferation, migration, and differentiation.

RESULTS: *In vitro* 2D study: all Mg^{2+}-treated groups presented improved expression of alkaline phosphatase activity, with 25mM $MgCl_2$ exhibiting superior mineral deposition assessed via Alizarin Red staining (Fig.1 A) and calcium quantification. We observed a dose-responsive inhibition in the formation of TRAP-positive multinucleated osteoclast-like cells with increasing concentration of Mg^{2+} (Fig. 1 B), and elevated extracellular Mg^{2+} significantly downregulating the expression of osteoclast markers TRAP and CTSK.

In vitro 3D study: we successfully produced a range of highly porous collagen composite scaffolds (Fig. 1 C). Mg^{2+}-modified scaffolds reduced the expression of osteoclast markers over time, demonstrating an effect in delaying osteoclast differentiation (Fig. 1 D).

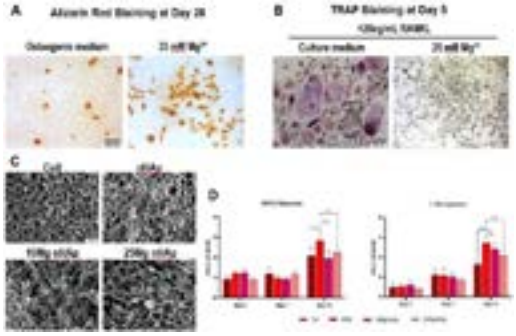

Fig. 1: A) MC3T3-E1 Alizarin Red staining at Day 28, B) RANKL-stimulated RAW 264.7 TRAP staining at Day5, C) SEM micrographs of collagen-composite scaffolds, and D) gene expression of osteoclasts markers of RANKL-stimulated RAW 264.7 cells seeded on collagen composite scaffolds.

DISCUSSION & CONCLUSIONS:
Our research to date demonstrates the potential of Mg^{2+} to concurrently enhance osteogenesis while inhibiting osteoclastogenesis *in vitro*, potentially introducing new strategies to develop bio-instructive biomaterials to repair metabolically compromised bone fractures.

ACKNOWLEDGEMENTS: This study has received financial support of Science Foundation Ireland, SFI/AMBER (17/RC-PhD/3477).

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The mechanisms of soft tissue toxicity in medication-related osteonecrosis of the jaw

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INTRODUCTION: Medication-related osteonecrosis of the jaw (MRONJ) presents as necrotic bone sections exposed via soft tissue lesions which fail to heal, and is without an effective treatment [1]. It is primarily caused by bisphosphonates (BPs), a group of drugs used to treat osteoporosis and cancers which have metastasised to bone. A variety of different processes have been linked to the development and progression of MRONJ, including soft tissue toxicity, which has been highlighted as important [2], both to development and patient quality of life. Hence, this study aimed to examine the effects of BPs on different cellular mechanisms within the oral mucosa, and determine any role in the soft tissue toxicity found in MRONJ.

METHODS: Human oral fibroblasts and keratinocytes were cultured in 2D monolayers and as 3D oral mucosa models using de-cellularised dermis as a scaffold, and treated with BPs. Assays were used to examine a variety of different mechanisms of viability and wound healing, including metabolic activity, migration, proliferation and adhesion, using cell culture, flow cytometry, histological and immunohistochemical techniques.

RESULTS: BPs were shown to affect cells of the oral mucosa at physiologically relevant concentrations in both 2D and 3D. BPs significantly affected apoptosis within these cells. Proliferation was reduced in both 2D monolayers (*Figure 1*) and within the epithelia of 3D oral mucosa models. BPs did not affect migration in an assay which controlled for proliferation. The development of oral mucosa epithelia was affected, as well as epithelial thickness.

DISCUSSION & CONCLUSIONS: The development of MRONJ is a complex process covering a wide variety of cellular mechanisms. This work demonstrates that BPs lead to thinner and disrupted epithelia, and hence why the oral mucosa lesions form and fail to heal within the MRONJ wound, and also why, as of yet, no effective MRONJ treatment has been developed. Interestingly, migration was not affected in our study despite being an oft-reported literature trend, however in studies which have not controlled for proliferation, suggesting the reduced wound healing capacity is due to viability and proliferation alone. Enabling the oral mucosa to heal and preventing exposure of the necrotic bone would slow disease progression and improve patient quality of life, reducing pain from everyday activities such as eating.

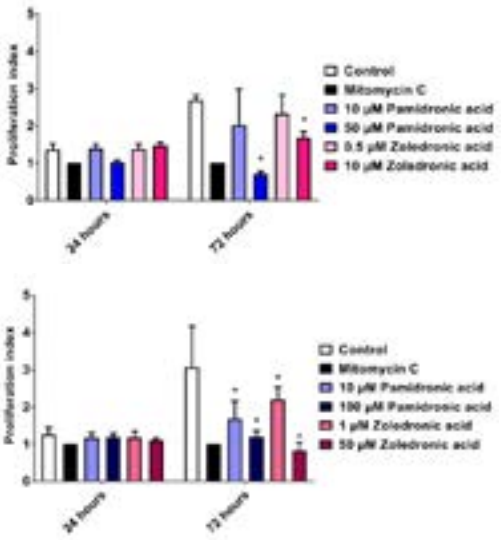

Figure 1: Human oral fibroblast (top) and immortalised human oral keratinocyte (bottom) proliferation in the presence of pamidronic acid or zoledronic acid, measured by CFSE flow cytometry proliferation assay.

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

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Evaluation of native nerve-derived extracellular matrix for peripheral nerve regeneration

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INTRODUCTION: The repair of peripheral nerve injuries is limited by current clinically available nerve guide conduits (NGCs), which do not provide adequate levels of regeneration compared to the gold-standard of a nerve autograft. Studies suggest that the use of individual extracellular matrix (ECM) components can improve nerve regeneration in NGCs (1), yet fewer studies have investigated the effect of a more native-like extracellular matrix as a scaffold for nerve regeneration, containing all the components present in a peripheral nerve. Therefore, in this project we aimed to develop a native, nerve-derived ECM to act as a scaffold within nerve guide conduits.

METHODS: To produce the native-like ECM, embryonic chick-derived dorsal root ganglia (DRG) were cultured on tissue culture plastic allowing the outgrowth of primary DRG neurones with supporting cells leading to the deposition DRG-derived ECM. The cellular components of the DRG-derived ECM were removed by a mild decellularisation protocol, resulting in a naturally-obtained decellularised ECM (dECM), maintaining features of the native peripheral nerve tissue. Neuronal NG108-15 cells were cultured for 1 week on the dECM surface and the morphology was compared to that of the same cells on control (TCP) to evaluate the potential of the dECM on nerve regeneration.

RESULTS: Successful decellularisation was evaluated by the removal of Dapi-stained nuclei, while ECM collagen content was evaluated by the Sirius Red assay. The dECM was then evaluated for nerve regeneration using a neuronal cell line (NG108-15) which showed significantly improved cell attachment and morphology on the dECM compared to control. The results highlight a potential for using naturally DRG-derived ECM in nerve regeneration (Fig 1).

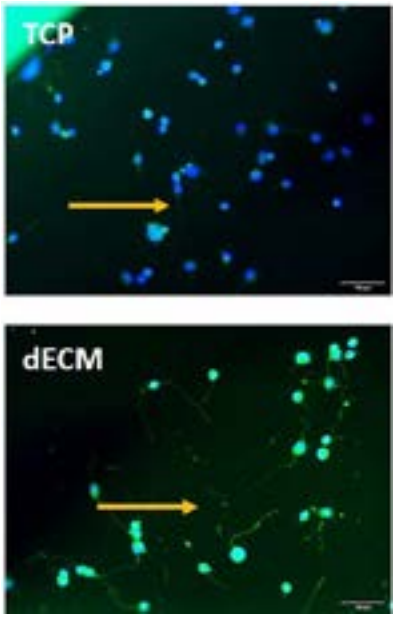

Figure. 1. Neuronal NG108-15 cells grown on the decellularised matrix (dECM) show increased neurite extension and cell attachment compared to TCP. Neurites highlighted by yellow arrow. Blue: DAPI stain for nuclei, Green: Phalloidin-FITC stain for F-actin filaments of neurites. Scale bar 200µm.

DISCUSSION & CONCLUSIONS: DRG can be successfully used to deposit ECM on which neuronal cells show improved morphology compared to control, thereby highlighting the potential for naturally-derived and nerve-specific ECM in nerve regeneration. Future studies are investigating the combination of the ECM with NGCs for *in vivo* evaluation of nerve repair.

ACKNOWLEDGEMENTS: EPSRC for funding.

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3D Bioprinting Whole Intervertebral Discs To Understand Development & Inform Regenerative Therapies

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INTRODUCTION: Back pain is one of the leading causes of disability, with an estimated 540 million sufferers worldwide. Degeneration of the intervertebral discs (IVD) is frequently implicated; however, treatment options remain limited and regenerative therapies are fast becoming essential. 3D bioprinting provides a powerful new tool for mimicking the structure and composition of complex tissues, such as the IVD; suitable cells and hydrogels for use in a bioprinted model of the IVD must therefore be identified and developed. This study aims to use bioprintable hydrogels to replicate the complex mechanical, biochemical and structural cues present within the IVD and to bioprint models of the developing, healthy, and degenerating IVD. It is hypothesised that by varying printed hydrogel mechanical properties (i.e. stiffness), composition (e.g. incorporating important extracellular matrix components such as laminin), and bioactivity (by presenting growth factors), healthier disc cell phenotypes can be maintained and the results used to inform IVD regenerative strategies.

METHODS: Immortalised human nucleus pulposus (NP) cells from the IVD's central NP region were suspended in blended alginate-collagen and alginate-collagen-laminin hydrogels using a protocol previously developed within the lab group[1]. Cell-laden gels were cultured for 21 days, then imaged using confocal laser scanning microscopy. Live/dead staining was used to ascertain cell viability. RNA was extracted and expression of COL1A1, COL2A1, ACAN, KRT8, KRT18, KRT19, CD24 & SOX9 analysed using qPCR. NP cell markers ACAN, HA and KRT8 were investigated using immunostaining (IHC/IF). Rheology was used to ascertain shear thinning and stiffness of the gels before bioprinting.

RESULTS: Rheological data confirmed that a wide range of physiologically relevant stiffnesses (1-100kPa) can be achieved, by varying either alginate or calcium chloride concentration. Shear thinning gels were used to create constructs with imprinted stiffness gradients. All blended hydrogels demonstrated high cell viability. Laminin was shown to encourage cell clustering. Immunostaining revealed substantial production of HA and ACAN, whilst qPCR demonstrated phenotypic responses to gel stiffness and composition.

DISCUSSION & CONCLUSIONS: The demonstration of stiffness gradients has made it possible to replicate an IVD with a 'soft' (~1kPa) NP-like region, a 'stiff' (~100kPa) annulus fibrosus-like (AF) region, and an 'NP-AF interface-like' region containing both NP and AF cells. Softer and stiffer variants of this template can additionally be created to specify 'healthy' and 'degenerate' IVDs. Protocols developed for the characterisation and analysis of 3D gel cultures will now be applied to bioprinted cell-laden gels of different stiffnesses and compositions; the discovery that laminin encourages clustering is a particularly interesting result that must be investigated further when applied to a model of the foetal IVD, since laminin is especially present at this early stage. The introduction of growth factors (TGFβ, GDF-5/6) is now of particular interest, since gel composition has been shown to influence NP cell phenotype using both PCR and immunostaining. The platforms developed here will ultimately be applied in bioprinting to optimise protocols for IVD regeneration using primary NP cells or stem cells.

ACKNOWLEDGEMENTS: Funding generously provided by UKRI (EPSRC & MRC) in support of the CDT programme in Regenerative Medicine.

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3D Bioprinting Tissue Engineered Meniscal Constructs

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INTRODUCTION: Meniscal injuries are the most commonly recorded knee injury by orthopaedic clinicians, affecting over 1.5 million people across Europe and the USA. Injury can greatly reduce knee joint mobility and quality of life, and often leads to osteoarthritis. Tissue engineering (TE) strategies have emerged in response to a lack of viable treatments for meniscal pathologies. However, the lack of TE constructs capable of mimicking the structural and functional organization of the native tissue whilst promoting the deposition of new extracellular matrix remains as one of the major bottlenecks in meniscal repair. 3D bioprinting allows for the deposition and patterning of biological materials with high spatial resolution. These parameters can be exploited to fabricate hybrid constructs through co-printing. The convergence of melt-electrowriting (MEW) and extrusion based hydrogel printing allows for micropatterning of fibrous meshes, with mechanical integrity, and regionally defined deposition of cell populations in hydrogel bioinks. This project aims to develop a truly biomimetic meniscal substitute, that replicates the structural and functional organisation of the meniscus capable of restoring knee articulation.

METHODS: Histological analysis was employed to investigate regional variations in collagen fibre orientation (picrosirius red) and matrix components proteoglycans (safranin-O/fast green), glycosaminglycans (alcian blue), elastin (Miller's) and vasculature (Masson's trichrome) across porcine meniscal tissue. 3D laser scanning (Einscan Pro) was employed to acquire external tissue geometries. The raw data produced 2D cross-sectional slices, processed into a 3D model using computer aided design (SolidWorks®). Suture tabs were added during the design element to increase ease of implantation to improve clinical translation. A 3D extrusion printed (3D GBIRE) meniscus was fabricated in polylactic acid (PLA). Cells isolated from inner and outer meniscus were cultured for 14 days in a blended alginate/ collagen bioink and viability assessed using a Live/Dead Assay.

RESULTS: Histological stains revealed regionally distinct variations in collagen fibre orientation and matrix composition. Isolation of cells from both inner and out regions of porcine meniscal tissue revealed morphologically distinct cell populations. 3D printing was able to accurately replicate meniscal anatomy (Fig 1.).

Figure 1: Sequential conversion from laser scanning fresh porcine meniscal tissue (A), CAD modelling (B-D), through to a fabricated 3D printed meniscus model (E).

Primary porcine meniscal cells remained viable in alginate/collagen hydrogels for 14 days, supporting its use in future bioprinting studies.

DISCUSSION & CONCLUSIONS: Meniscal tissue characterisation reveals distinct regional variations in tissue architecture and cell populations. Future work within this project will involve further characterisation of tissue and cellular phenotype to inform the design, material and cell selection. Fabrication of a 3D printed meniscus highlights the potential to print patient specific meniscal implants. Future work will involve manipulating fibre orientation to grossly mimic native meniscal tissue collagen architecture which relates closely to its mechanical functioning. The blended alginate/collagen hydrogel will be encapsulated with cells from inner and outer regions of the meniscus respectively and co-printed regionally. Achieving the intricate fibre architecture and zonally distinct variations in cell and matrix deposition will highlight the ability to fabricate a highly complex tissue engineered construct capable of restoring knee articulation in those with meniscal pathologies.

ACKNOWLEDGEMENTS: This work was undertaken as part of the UK Research and Innovation (UKRI)-funded CDT in Advanced Biomedical Materials.

Collagen and alginate hydrogels support re-differentiation of *in vitro* dedifferentiated ovine chondrocytes

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INTRODUCTION: Focal cartilage defects are a common knee problem with a high prevalence (20%) among people of all ages [1]. Due to its avascular nature cartilage has limited ability to self-repair and defects can lead to pain and to osteoarthritis in the long term. To repair these defects, autologous chondrocyte implantation (ACI) has been used for two decades with successful surgical outcomes, however, one of its main limitations is chondrocyte (CHs) de-differentiation during *in vitro* expansion [2]. Here we isolated ovine CHs and studied their de-differentiation from P1 to P4 in 2D culture. We then encapsulated dedifferentiated CHs into collagen and alginate (col/alg) hydrogels and hypothesised that these 3D scaffolds would support the cells re-differentiation to CHs in the absence of chondrogenic inducers.

METHODS: Primary CHs were isolated from ovine articular cartilage fragments by incubation with type VIII collagenase. CHs were expanded in 2D and analysed for gene expression and morphological changes at P1 and P4. Dedifferentiated CHs were encapsulated into col/alg hydrogels. At day 1, 7 and 14 a Live/Dead assay was performed. At day 7, morphology was assessed using Phalloidin staining and production of collagen type II (Coll II) and aggrecan was assessed by immunofluorescence. Expression of chondrogenic genes was evaluated at mRNA level using RTqPCR.

RESULTS: From P1 to P4, CHs expanded in 2D culture lose their rounded morphology (aspect ratio of 1.5) to acquire a spindle shape with abundant stress fibres (Fig 1A), they present progressive loss of chondrogenic markers (SOX9, SOX5, SOX6, COL2A1) and the over-expression of hypertrophic markers (COL1A1). Dedifferentiated CHs embedded into col/alg hydrogels remain viable up to day 14 (Fig. 2); furthermore they started to aggregate and regain the typical rounded morphology and producing aggrecan and Coll II (Fig. 3).

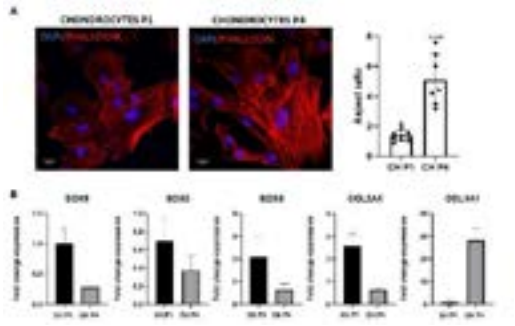

Fig. 1: Dedifferentiation of CHs from P1 to P4. (A) Phalloidin and dapi staining of CHs P1 and CHs P4. (B) The expression of typical chondrogenic markers evaluated at mRNA level presented as fold change using the 2−ΔΔCt method.

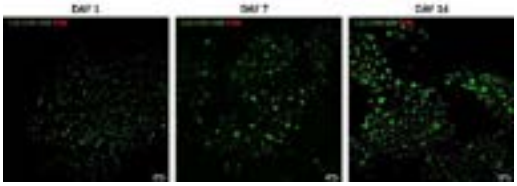

Fig. 2: Dedifferentiated chondrocytes viability in col/alg hydrogels. Live and dead staining using calcein-am and ethidium hodomodimer-1 of dedifferentiated CHs embedded into col/alg hydrogels.

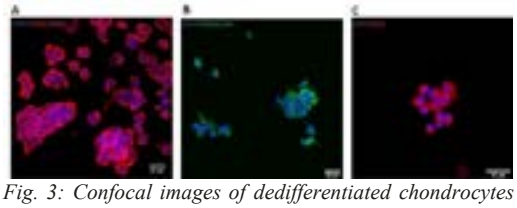

Fig. 3: Confocal images of dedifferentiated chondrocytes embedded into hydrogels at day 7. Immunofluorescence of (A) phalloidin and dapi staining. (B) Dapi and aggrecan staining (C) dapi and Coll II staining.

DISCUSSION & CONCLUSIONS: This work showed that CHs in col/alg hydrogels were viable and aggregated producing extracellular matrix from day 7 without any external growth factors. Further gene expression analysis will confirm the ability of hydrogels to support the re-differentiation of *in-vitro* expanded CHs potentially enhancing success rate of ACI procedures.

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Facile Techniques to Induce Controllable Topographic Patterns on PDMS for Epithelial Tissue Growth

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INTRODUCTION:

In tissue engineering and regenerative medical applications, the demand for *in vitro* modelling and culture systems has drastically increased. In order to produce truly biomimetic systems, tissue structural replication is a vital consideration and often presents practical challenges. Whilst many applications opt towards 3D scaffolds, certain tissue structures such as those found in epithelia, require less of a bulk scaffold approach but surface topography replication. Epithelia tend to present undulating or crypt structures vital to the physiological function of the tissue and in adult stem cell population maintenance in their niches. These structures *in vivo* form part the self-regulating cell growth guidance mechanisms which underpin tissue regeneration and maintenance. Polymer surface wrinkling has provided the means to create such topographies[1], as the fabricated undulating surface closely matches to epithelial surface structures. Currently moulding and lithography techniques are used to generate undulating topographies in PDMS for cell culture, however these techniques require pre-made masters, often employing expensive materials and methods to produce. Additionally, such methods rarely allow for substrate tuneability or control of topography formation. The study has optimised the acid oxidation and low temperature plasma techniques for wrinkle formation. These techniques are considered lower cost alternatives for producing wrinkled substrates for use in topographical based cell culture systems which control cell behaviour and distribution, particularly for epithelial tissues.

METHODS: Polydimethylsiloxane substrates were mixed to 5% curing agent in elastomer and cast at 85°C for 2 hours. Wrinkling was generated by two methods: 1) Exposure of pre-strained (10% stretch) substrate to a partially thermal-decomposed mixture of sulfuric and nitric acids in a 3:1 ratio with subsequent substrate neutralisation in sodium bicarbonate solution, 2) Exposure of pre-strained substrates to low temperature oxygen plasma *in vacuo*. Uniaxial wrinkling forms across the surface upon relaxation of the applied strain.

RESULTS:

Both the substrate wrinkling methodologies influence produce surface wrinkling, however there is a difference in presentation and surface morphology between the two methods. Wrinkles generated by plasma treatment have a greater frequency of topography features leading to a decreased crypt width compared to the acid oxidised surfaces.

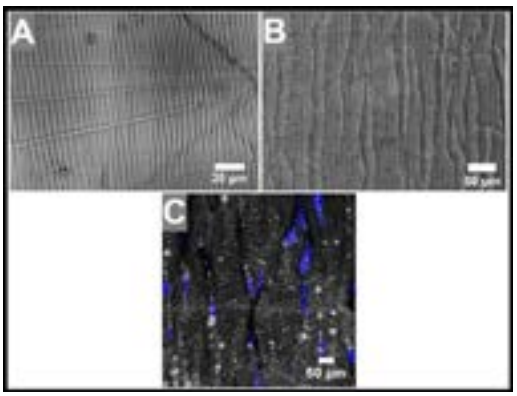

Fig. 1: Examples of various wrinkle topographies produced by both methods; A) Plasma treatment, B) Acid Oxidation, C) HEK293 cells stained with DAPI (blue) on acid oxidised PDMS substrate.

DISCUSSION & CONCLUSIONS: The presented topographies of substrates have been demonstrated to be suitable for the construction of topographical substrate systems wherein a degree of cell behaviour control has been demonstrated and the biocompatibility of these surfaces has been sufficiently optimised to enable the culture of cells on these surfaces.

ACKNOWLEDGEMENTS: This project is funded by the EPSRC CDT programme for Regenerative Medicine

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Towards A Computational Model Of Vascular Network Formation In A Hydrogel To Aid The Fabrication Of Engineered Tissues

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INTRODUCTION: Rapid vascularisation of engineered tissues once implanted *in vivo* is essential to ensure sufficient nutrient supply to support therapeutic cells [1]. Prevascularisation by seeding with endothelial cells (ECs), which can form capillary-like structures *in vitro* and anastomose with host vasculature upon implantation, is one such promising method [1].

Incorporating many experimental variables and based on a balance of chemical and mechanical cues, *in vitro* EC network formation is well placed to be explored computationally with use of mechanistic mathematical models [2]. Here, we demonstrate an adaptable mathematical model for vascular network formation in a 3D hydrogel, integrating global features such as construct geometry, environmental conditions, and interstitial flow, with key chemical and mechanical cell mechanisms that lead to network formation [2].

METHODS: Based on a multiphase model framework [3], a set of coupled partial differential equations (PDEs) describe the temporal and spatial evolution of the EC density, extracellular matrix density, oxygen and VEGF concentrations. Key cell mechanisms include autologous chemotaxis, oxygen-dependent VEGF production, and cell-matrix interactions mediated by traction and matrix stiffness.

The model, implemented and solved in Python, remains adaptable to hydrogel properties, additional cell types, and culture conditions.

RESULTS: Combining data from literature, and utilising computational methods including sensitivity analysis and parameter optimisation, we identify parameter sets under which capillary-like structures form. Metrics such as vessel length, area, and network complexity, enable validation of the computational model against available *in vitro* data.

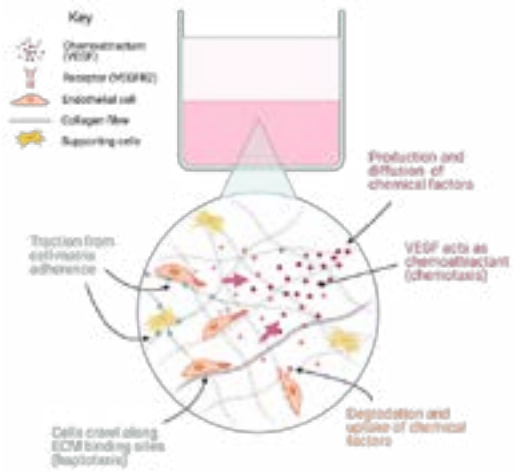

Fig. 1: Key cell mechanisms that lead to vascular network formation in vitro. Created with BioRender.com.

DISCUSSION & CONCLUSIONS: We demonstrate a flexible model that allows us to elucidate the benefits of two mechanisms frequently neglected, but with increasingly recognised significance for functional endothelial network formation: culture under physiological hypoxia, and low levels of interstitial flow/shear stress [1, 4]. With further refinement of the PDE model, alongside tailored *in vitro* experiments, it is hoped that simulation predictions will aid and inform the culture of functional, perfusable vascularised engineered tissues for clinical use.

ACKNOWLEDGEMENTS: This work was supported by an EPSRC studentship EP/R512400/1 to GFAB and grant EP/R004463/1 to RJS and JBP.

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Incorporation of Retinyl Acetate into PCL Electrospun Scaffolds for Antioxidant Delivery

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INTRODUCTION: More than 700,000 new diagnoses and 350,000 deaths are associated with head and neck cancers annually.[1] The process of radiotherapy to treat the cancer can also damage otherwise healthy tissue, inducing adverse effects. With head and neck cancers, such effects include detrimental damage to the salivary glands, with the majority of patients who receive treatment developing permanent salivary gland dysfunction.[2] To date there is no permanent cure. Tissue engineered scaffolds can be used to support repair in a damaged tissue environment. High levels of reactive oxygen species (ROS) are a well-established indicator of oxidative stress within a cell and its surrounding environment. Recently, the antioxidant properties of vitamins have been exploited as a method of reducing the levels of ROS.[3] Vitamin A and its derivatives, such as retinyl acetate (RA), also exhibit these antioxidant properties.[3,4]
Herein, the incorporation of RA into scaffolds and its effect as an antioxidant delivery system in a ROS environment for salivary gland regeneration is studied.

METHODS: 0.1 % and 0.5 % RA (w/v) was incorporated into 10 % (w/v) PCL in HFIP. 4.0 mL of each solution was electrospun at a rate of 1.0 mL/h to produce randomly aligned fibre scaffolds. 10 % PCL in HFIP was used as a control. The morphologies of the scaffolds were observed using SEM imaging and the mechanical properties were measured by tensile testing. Culture of HSG (HeLa derivative) salivary gland cells on scaffolds were measured over 14 days for cell responses. A hydrogen peroxide (H_2O_2) assay was used to determine the antioxidant properties of the scaffold.

RESULTS: RA was successfully incorporated into PCL electrospun scaffolds. As shown in Figure 1, the fibres produced had similar morphologies. The properties of the scaffolds are given in Table 1. Addition of RA into the scaffolds was shown to have minimal effect on fibre morphology and diameter size. Scaffolds were shown to support the survival and proliferation of HSG cells and elicit antioxidant properties.

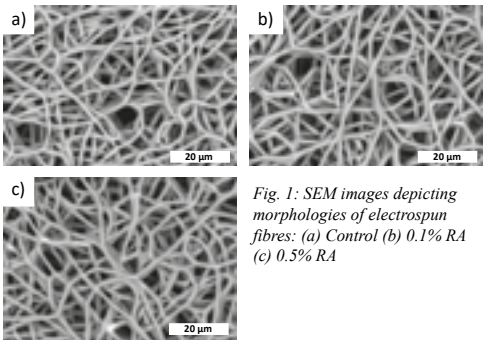

Fig. 1: SEM images depicting morphologies of electrospun fibres: (a) Control (b) 0.1% RA (c) 0.5% RA

Table 1. Scaffold Properties

	10 % PCL (Control)	0.1 % RA 10 % PCL	0.5 % RA 10 % PCL
Fibre Diameter (µm)	1.32 ± 0.12	1.54 ± 0.14	1.44 ± 0.10
Young's Modulus (MPa)	15.39 ± 6.16	14.04 ± 4.27	11.43 ± 8.90

DISCUSSION & CONCLUSIONS: Based on these initial results, it can be seen that the successful incorporation of RA into electrospun fibres can reduce the level of H_2O_2. This provides encouraging evidence that antioxidants have potential in regulating the environment in the salivary gland post cancer treatment, highlighting their potential as a viable therapeutic approach.

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Elucidating the effects of varying laminin emulsions in Polycaprolactone electrospun fibres

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INTRODUCTION: 10% of the world population suffers from kidney diseases[1]. As today's treatment options fall behind the need, tissue engineering has come forward to create alternative approaches for regenerative treatment. One of the corners in tissue engineering is scaffolds that can mimic the natural environment for cells to function as desired. Polymeric electrospun scaffolds have been widely investigated yet lack of biomolecular signals directs researchers to hybrid scaffolds[2]. In this study we fabricated hybrid scaffolds with varying laminin concentrations in polycaprolactone (PCL) via emulsion electrospinning to investigate the cell response for kidney tissue engineering.

METHODS: Briefly, 14% PCL and surfactant (Span80) in hexafluoroisoproponal (HFIP) were mixed with differing concentration of aqueous laminin solution and electrospun. Spinning parameters were changed to obtain similar fibre diameter to eliminate size effect[3]. RC-124 kidney cells were then cultured on the scaffolds up to 14 days. Morphological, mechanical and biochemical analysis were carried out.

RESULTS: Scaffolds seen in Figure 1 were successfully manufactured with similar fibre diameter ranging from 0.69 to 0.74 µm (Table 1). Seeded cells maintained survival up to 2 weeks and even penetrated into the hybrid scaffolds. 20% laminin scaffold shows higher DNA content in all time points indicating higher cell attachment. Similarly, mechanical properties of scaffolds did not change until 20% laminin concentration (Table 1).

DISCUSSION & CONCLUSIONS: These findings point out that increasing concentration of laminin in PCL fibre effects kidney cells functions. Further investigation is required for long term effects of the protein release from fibres on cell behaviour. The results highlight the importance of investigating hybrid scaffolds for kidney tissue engineering.

Table 1: *Fibre diameter and Young's modulus of the scaffolds.*

	Fibre diameter (µm)	Young's modulus (MPa)
0% Laminin	0.69 ± 0.27	1.80 ± 0.14
%5 Laminin	0.74 ± 0.28	1.94 ± 0.33
%10 Laminin	0.71 ± 0.27	1.86 ± 0.40
%20 Laminin	0.71 ± 0.27	1.28 ± 0.13

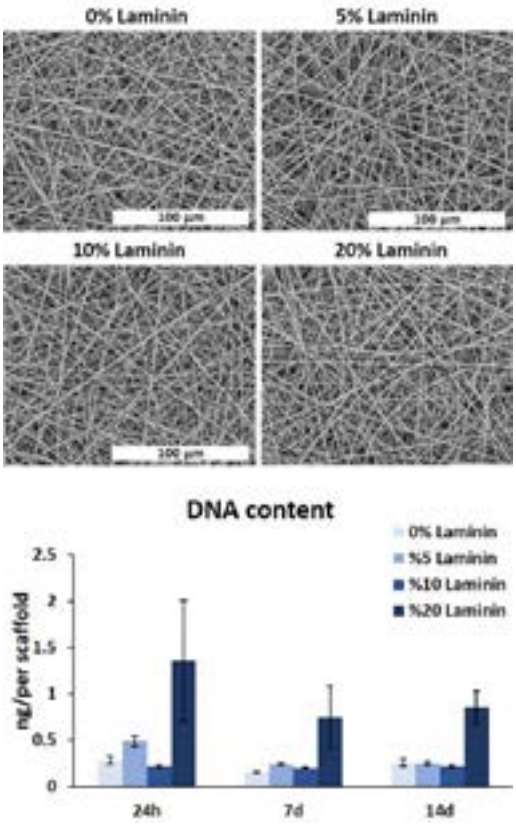

Fig. 1: *SEM images of the scaffolds (top) and DNA quantification of kidney cells on the scaffolds up to 14 days (bottom).*

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Reduced oxygen culture promotes proliferation and a mucin-producing phenotype in COPD distal airway stem cells

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INTRODUCTION: Lung diseases present a significant worldwide health burden with considerable associated mortality and morbidity. Frequently they have no curative therapies and limited symptomatic treatments, identifying them as strong, prospective regenerative medicine targets. The sourcing of epithelial cells and the creation of representative disease models has proved challenging, although progress has been made in culturing airway basal cells, information on the culture of more distal lung cells remains scarce.

METHODS: Distal lung tissue was obtained from a human donor with Chronic Obstructive Pulmonary Disease (COPD) undergoing lung volume reduction surgery. Tissue was minced and digested overnight. Cells were plated on type I collagen-coated tissue culture flasks and cultured in cFAD medium with rock inhibitor supplementation[1] in either a 2% or 21% O_2 environment. Cell proliferation was monitored and the resulting cells were characterised by immunofluorescence for vimentin, smooth muscle actin (SMA), pan-cytokeratin, TP63, cytokeratin 5 and E-cadherin. Cells were differentiated using air-liquid interface (ALI) culture with measurement of trans-epithelial electrical resistance (TEER) weekly and immunofluorescence and qPCR of differentiation markers CC10, β IV tubulin, Mucin5B and Mucin 5AC. ALI cultures were stained histologically with Alcian blue/periodic acid Schiff (AB/PAS) for mucins.

RESULTS: Isolated cells were colony forming, epithelial in appearance and predominantly expressed the epithelial markers Pan-cytokeratin and E-cadherin. Further, the epithelial cells expressed TP63 and cytokeratin-5 suggesting a distal airway stem cell (DASC) identity. Initial growth rates were similar in both O_2 levels but cells cultured in 21% O2 underwent growth arrest at 30 population doublings (PD) while 2% cultures continued to proliferate beyond 40 population doublings. At ALI cells had increased TEER (≥350 Ω.cm^2) and developed visible motile cilia. ALI cultures stained positively for CC10 and β IV tubulin. 2% O_2 cultures had a qualitative reduction in cilia compared to 21% O_2 (Fig. 1A). Both 2% and 21% cultures stained strongly with AB/PAS (Fig. 1B), 2% cultures had a trend for higher expression of *MUC5B* ($p \leq 0.05$) and *MUC5AC* at the gene level.

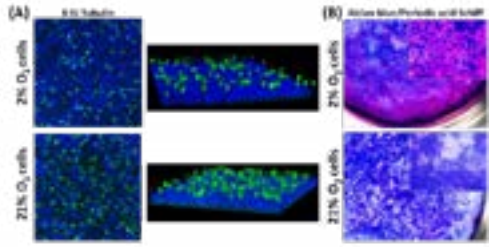

Fig. 1: Mucociliary differentiation at ALI. (A). Cilia stained positively with β IV Tubulin (B) AB/PAS staining of mucins.

DISCUSSION & CONCLUSIONS: We have successfully isolated and culture-expanded TP63 and cytokeratin 5 positive DASCs from the lung tissue of a COPD human donor, with reduced oxygen culture resulting in an enhanced proliferation capacity. We have demonstrated the multipotential differentiation capacity of the DASCs with CC10 progenitor cells, β IV tubulin and mucin positive cells representing a distal, bronchiolar phenotype. Extensive mucin staining was present, this was further enhanced by culture expansion of the cells in a reduced O_2 environment suggesting a key role for hypoxia, both in the underlying disease mechanism of COPD and in the *in vitro* reproduction of disease models.

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