British Society for Matrix Biology

Liverpool, 13-14th of September 2022

Maritime Museum

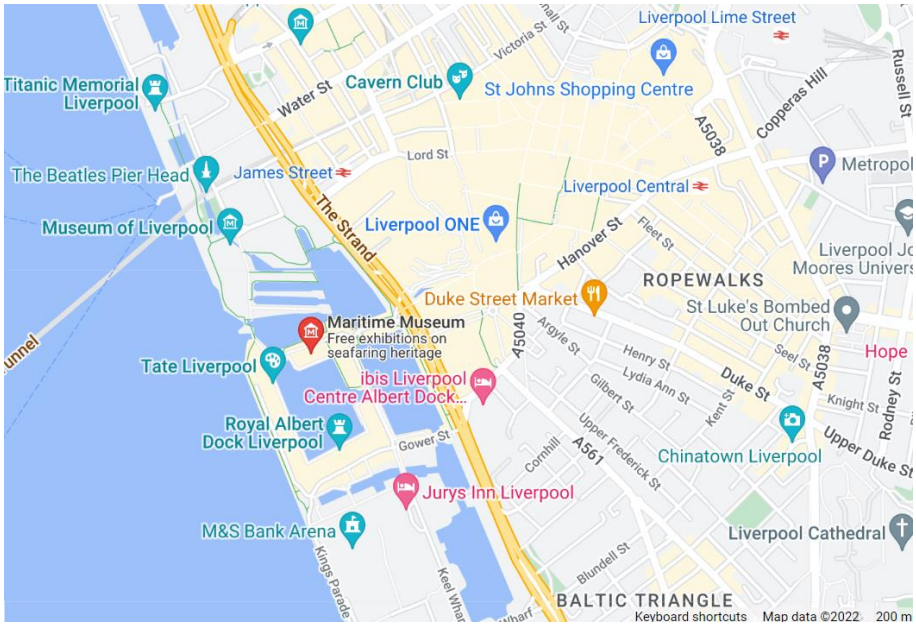
The Matrix in Development

VENUE:

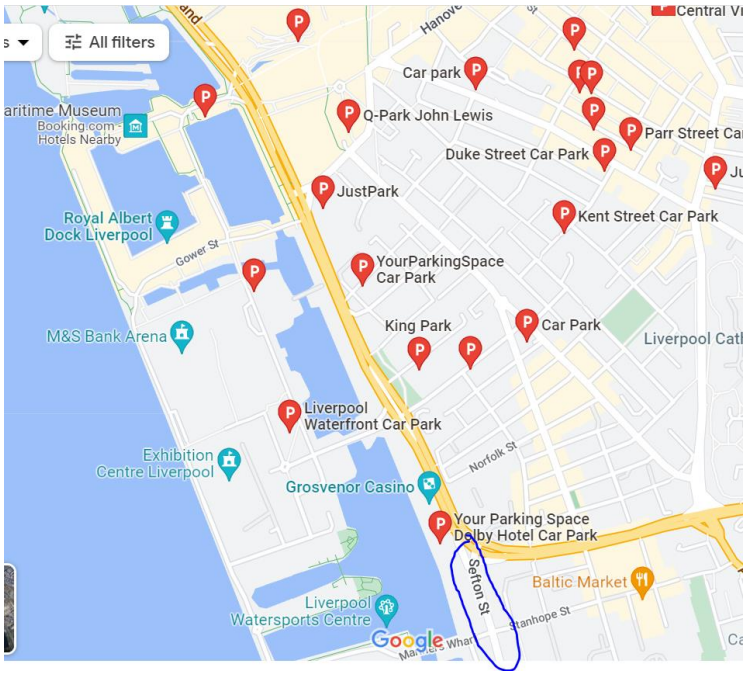
Maritime Museum, third floor;

Located in: Royal Albert Dock Liverpool; Address: Royal Albert Dock, Liverpool L3 4AQ.

https://www.liverpoolmuseums.org.uk/maritime-museum#section--getting-here



Multiple car parks are located nearby; for free car park (road side), I often try Sefton street. But you may need to walk a little to get to the venue (at your own risk).



PROGRAMME

Monday 12th September: Early career researchers pre-meeting

1.30-2.30pm: registration

2.30-5pm: Career workshop; guest speakers include

Dr Mittal Shah (Principal Translational Scientist NovalGen)

Dr Sarah Stevenson (medical writer at Delta Kn)

Dr Eamon Dubaissi (Research Staff Developer, Prosper: Unlocking postdoc career potential)

5-7pm: Networking social event (The Pump House).

Tuesday 13th September: start 12.30pm

11.30 – 12.30pm: Registration; lunch

12.30 -12.40pm: Opening remarks

12.40 – 2pm: Session 1: From stem cells to organs; *Chairs: Dr David Wilkinson*

Dr April Craft (30 min): *"Developmental chondrogenesis using iPSCs: a model to study the role of the ECM in differentiation and disease"*

Short talk 1 (10 min): Paul Humphreys, University of Manchester*, "Optogenetic Control of BMP Signalling to Drive hPSC Chondrogenesis"*

Short talk 2 (10 min): Emma Barker, University of Nottingham, *"Stem Cell Models of Glycosaminoglycan Developmental Disease"*

Dr David Turner (30min): *"Understanding embryonic development using gastruloids"*

2 – 3.30pm: Coffee and poster sessions; AGM

3.30 – 4.50pm: Session 2: Matrix composition and development; *Chairs: Dr Kevin Hamill, Dr Francesca de Sousa Brito*

Prof Cathy Merry (30 min), "*Glycosaminoglycans, critical roles in early development"*

Short talk 3 (10 min): Samuel Robert Moxon, *"A 3D Bioprinted Model of the Intervertebral Disc for Disease Modelling Applications"*

Short talk 4 (10 min): Angus Nichols*, "Building and turning over the Drosophila Basement membrane: dissecting formation, function and plasticity of extracellular matrix during development"*

Prof Florence Ruggiero (30 min): "*Muscle-derived collagens as key players of neuromusculoskeletal development: what a tiny fish can teach us".*

4.50 -5.30 pm: John Scott lecture: Dr Joan Chang. Chair: Prof Andy Pitsillides

7pm – Dinner (Hilton)

Wednesday 14th of September:

9-10.10am: Session 3: Organ development; *Chairs: Dr Kasia Pirog, Ms Emily Shorter*

Dr Emily Noel (30 min): *"Timely establishment of an apical Laminin sheath maintains ventricular wall integrity during heart development*

Short talk 5 (10 min): Alina Kurjan, Oxford University *"Defining the Transcriptional Landscape of Developing Human Tendons"*

Dr Claire Clarkin (30 min) – online *"Multiscale characterisation of angiogenic matrix signatures in skeletal health and disease"*

5 min break

10.15 – 11.05am: Session 4: BSMB open meeting; *Chairs: Dr Joan Chang, Dr Agnieszka Turlo.*

Dr Sarah Snelling (30 min): *"Current themes and knowledge in human tendon development" – Mathew Baldwin*

Short talk 6 (10 min): Sara Ibrahim AlSalhi, University of Liverpool, *"Transcriptional elements driving MMP13 in development"*.

Short talk 7 (10 min): Salvatore Santamaria, Imperial College London, *"Mapping proteolytic events in extracellular matrix proteins using a label-free quantitative proteomics approach"*

11.05am – 12.05pm: Coffee and Posters

12.05-1.15pm Session 5: Epigenetic influences; *Chairs: Ms Emily-Jayne Clarke, Dr Yalda Ashraf Kharaz.*

Dr Ioannis Kanakis (30 min): *"Low protein intake during reproduction: effects on maternal and offspring musculoskeletal system"*

Short talk 8 (10 min): Shijian Fu, University of Liverpool, *"RNA binding protein HuR regulate osteoclast induction capacity of MG63 cells*"

Prof Audrey McAlinden (30 min): *"The miR-181a/b-1 cluster enhances skeletal cell metabolism and bone matrix formation"*

1.15 – 1.25pm: Poster/presentation prizes and closing remarks

ABSTRACTS

SHORT TALKS

P1: Optogenetic Control of BMP Signalling to Drive hPSC Chondrogenesis.

Paul Humphreys[1], Fabrizio Mancini[1], Steven Woods[1], Julieta O'Flaherty[1], Marco Domingos[2], Susan Kimber[1].
[1]Division of Cell Matrix Biology and Regenerative Medicine, The University of Manchester, Manchester, UK. [2]Department of Mechanical, Aerospace and Civil Engineering, The University of Manchester, Manchester, UK.

Introduction:
Visible light is increasingly being used to control cellular function and physiology through a technology called optogenetics. Optogenetic tools typically consist of a photoreceptor chimerised to a signalling molecule of interest to render its activity light sensitive. Optogenetic approaches enable dynamic perturbation that can be fine-tuned through modulation of frequency, wavelength and intensity. Incorporation of optogenetics within developmental models allows exploration into how subtle variation of cell signalling mechanisms can influence cell fate and phenotype.

Materials and Methods:
We generated an optogenetic Bone Morphogenetic Protein (BMP) system (optoBMP) to manipulate chondrogenic differentiation of human pluripotent stem cells (hPSCs). The OptoBMP system consisted of modified BMPR1B/ALK6 and BMPR2 receptors, in which the intracellular regions of the receptors were membrane anchored with a myristoylation domain at the N-terminus and a light sensitive Light-Oxygen-Voltage (LOV) sensing domain chimerised at the C-terminus. An optoBMP system transgene was inserted into the AAVS1 locus of hPSCs through CRISPR/Cas9 gene editing (optoBMP-hPSCs). Supplementation of BMP growth factors within an hPSC chondrogenic differentiation protocol was replaced with an optimised regime of blue light stimulation. The effect of optogenetic manipulation upon hPSC chondrogenic differentiation was characterised through protein and transcriptional analyses.

Results:
Illumination of optoBMP-hPSCs resulted in activation of canonical BMP signalling through phosphorylation of SMAD1/5/8 and initiation of BMP-related transcriptional activity. RNAseq and qPCR analyses indicated that substitution of BMP growth factors for light illumination could drive hPSC chondrogenic differentiation, resulting in significant upregulation of key chondrogenic genes (SOX5,6,9, COL2A1, ACAN) and enrichment of GO terms associated with developmental chondrogenesis (skeletal system morphogenesis, cartilage development). Optogenetic manipulation resulted in significantly higher expression of specific genes when compared to BMP growth factor addition, including SOX5,6 and COL2A1. Furthermore, analysis of non-canonical MAPK signal transduction indicated that optoBMP activation resulted in significantly increased MAPK pathway activity compared to BMP growth factors.

Discussion:
Our findings indicate that the optoBMP system can be utilised to drive chondrogenic differentiation of hPSCs in lieu of BMP growth factors. Furthermore, optoBMP activation enhanced expression of key cartilage matrix genes, such as COL2A1 and ACAN, which indicates that optogenetic-mediated signal transduction through BMPR1B is a promising approach to tissue engineering of hPSC-cartilage constructs.

P2: Stem Cell Models of Glycosaminoglycan Developmental Disease.

Emma Barker[1], Sara Pijuan-Galitó[1], Matteá Finelli[2], Catherine Merry[1].
[1]Stem Cell Glycobiology, University of Nottingham, Nottingham, United Kingdom. [2]Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom.

Introduction:
Heparan sulphate (HS) is a key component of the extracellular matrix and can bind to proteins, such as the fibroblast growth factor family (FGFs), to regulate critical biological processes. Sulphated residues within HS chains control their location, function and affinity for specific ligands. Previously, lack of the HS 2-O-sulphotransferase (HS2ST1) enzyme in mice was shown to be embryonic lethal due to skeletal abnormalities and kidney agenesis. HS2ST1-/- mouse HS lacks 2-O-sulphation, has increased 6-O- and N-sulphation and therefore retains an approximately normal charge distribution across the chain despite lacking a sulphation motif common to many protein-binding domains. Using HS isolated from the HS2ST1-/- mouse, the affinity for FGF-1 and FGF-2 was found to be decreased, however HS2ST1-/- cells remained responsive to these growth factors. Recently, four human patients with alterations in the HS2ST1 gene have been investigated. Patients (<14Yrs) present with diverse genetic alterations in HS2ST1, and have skeletal and renal abnormalities, including absent kidneys.
Materials and Methods:
Media and cell lysate of cultured patient fibroblasts were collected and the HS isolated and fluorescently labelled. The disaccharide composition of the HS of patients was then analysed by RP-HPLC. Human induced pluripotent stem cells (hiPSCs) were used to knockout HS2ST1 using CRISPR-Cas9. Confirmation of knockout, and loss of the 2-sulphated HS disaccharide, was carried out by flow cytometry and immunostaining using a HS antibody (10E4) and an antibody that preferentially binds the 2-sulphation motif (AO4B08). hiPSCs with homozygous and heterozygous HS2ST1 knockouts were then differentiated to neural precursor cells and definitive endoderm. Immunostaining, flow cytometry and quantitative reverse transcription PCR (qRT-PCR) were used to assess the differentiation potential of the cells compared to wild type hiPSCs.
Results:
HS disaccharide analysis of patient fibroblasts revealed the HS to have a similar composition to HS2ST1-/- mice, including a complete loss of 2-O-sulphation and an increase in N- and 6-O-sulphation. HS2ST1-/- hiPSCs show positive staining for 10E4 and negative staining for AO4B08 in both flow cytometry and immunostaining. Heterozygous knockouts show some AO4B08 staining but the levels are not as high as wild type controls, but not as low as negative controls (HS2ST1-/- CHO cell line). Initial studies show that knockout cell lines differentiative to neural precursor cells similar to wild type hiPSCs.
Discussion:
This data shows that patients are able to survive without the HS 2-sulphation motif, contrary to what was previously hypothesized. This finding led to the creation of a hiPSC knockout cell line. Confirmation of the creation of HS2ST1 knockout hiPSCs provides an invaluable tool that can be used in structure/function studies to better understand the mechanistic basis of the observed phenotype. In addition to flow cytometry and immunostaining, compositional disaccharide analysis will be carried out to confirm to the loss of 2-sulphated HS disaccharides and determine whether disaccharides display increased sulphation at other locations, as in mice and humans. Further works is required to confirm initial findings of the neural differentiation potential of HS2ST1-/- and HS2ST1+/- cell lines. Furthermore, it is important to assess the differentiation potential to mesoderm lineages.

P3: A 3D Bioprinted Model of the Intervertebral Disc for Disease Modelling Applications.

Samuel Robert Moxon[1,2,5], Zachary McMurran[1], Marco Domingos[2,3,5], Julie Gough[3,4,5], Stephen Michael Richardson[1,2].
[1]School of Biological Sciences, University of Manchester, Manchester, UK. [2]Advanced Materials in Medicine, University of Manchester, Manchester, UK. [3]School of Engineering, University of Manchester, Manchester, UK. [4]School of Natural Sciences, University of Manchester, Manchester, UK. [5]Henry Royce Institute, University of Manchester, Manchester, UK.

Introduction:
The intervertebral disc (IVD) aids the movement of the spinal column, providing vertebra with support against loads. It is comprised of a soft, polysaccharide-rich nucleus pulposus (NP) surrounded by an annulus fibrosus (AF) of aligned, stiff, collagenous matrix. Degeneration of the disc is a common cause of lower back pain and can result in permanent disability. The development of therapies is often hampered by a lack of in vitro models that allow for the accurate recapitulation of the tissue microenvironment. This study aims to investigate if 3D bioprinting could be employed to fabricate analogues of the IVD for studying tissue homeostasis and disc disease modelling.
Materials and Methods:
Biphasic hydrogel constructs were fabricated via suspended layer additive manufacturing (SLAM) containing NP regions of gellan gum polysaccharide (gellan) and AF regions of type I collagen. Human NP and AF cell lines were incorporated and cultured for 28 days. Constructs were analysed with atomic force microscopy (AFM) to evaluate gel microstructure and biomechanics. Phalloidin staining was used to evaluate cell morphology. Cell tracking dyes were employed to evaluate cell migration and fluorescent histology was utilised to screen for extracellular matrix depositions.
Results:
Constructs were successfully fabricated containing a defined NP and AF. AFM revealed that bioprinting facilitated the alignment of collagen fibres in the AF. Live cell tracking highlighted the incorporation of NP and AF cells into the two tissue regions. The embedded cells exhibited in vivo-like morphologies (rounded NP cells, elongated AF cells). Actin staining revealed alignment in the AF cells in parallel to the collagen fibres. Fluorescence staining highlighted hyaluronic acid (HA) deposition by NP cells over 28 days.
Discussion:
The bioprinted IVD tissue analogue recapitulated structural, morphological and biological components present in the native tissue on macro and micro scales. The construct exhibited key structural components of native tissue with a central, polysaccharide-rich NP surrounded by organised, aligned collagen fibres. Cell tracking, actin and matrix staining demonstrated that embedded cells exhibited morphologies and phenotypes analogous to in vivo with elongated, aligned AF cells and spherical NP cells depositing HA. The structure contained a defined NP-AF interface, mimicking the highly regulated structure of the IVD.

P4: Building and turning over the *Drosophila* Basement membrane: dissecting formation, function and plasticity of extracellular matrix during development.

Angus, Nichols[1], Besaiz J., Sánchez-Sánchez[1], María-Carmen, Diaz-de-la-Loza[1], Stefania, Marcotti[1], Mychel RPT, Morais[2], Rachel, Lennon[2], Eduardo, Serna-Morales[3], Liyamu, Ma[1], Maciej, Jedraszak[1], Brian, Stramer[1].
[1] Randall Centre for Cell & Molecular Biophysics, King's College London, London, England. [2]Division of Cell Matrix Biology & Regenerative Medicine, University of Manchester, Manchester, England. [3] Francis Crick Institute, London, England.

Introduction:
Basement membranes (BMs) are a specialised form of extracellular matrix (ECM) with a thin, sheet-like architecture. These sheets, ubiquitous within multicellular animals, act as extracellular scaffolds providing structural integrity and organisation to tissues as well as assisting in cell signalling and migration. Type IV collagen (ColIV) polymer networks are a key BM component providing rigidity and tensile strength, but our knowledge of their formation is largely derived from in vitro studies. We also lack insight into how BM stability and turnover rate changes as rapidly growing tissues develop into stable adult organs. These knowledge gaps impede our understanding of how BM stability and plasticity changes in genetic and acquired disease.
Materials and Methods:
We will exploit *Drosophila* as a genetically tractable model to dissect key residues essential to BM formation and stability by characterising two series of ColIV mutations: a set of truncations of the 7s domain believed to be essential for ColIV cross-linking, and a series of glycine substitutions analogous to those underlying human BM disease. To study BM turnover, we are developing a novel fluorescent ColIV timer approach. Furthermore, by establishing a method to analyse isolated insoluble ECM proteins with Mass spectroscopy, we shall compare relative BM solubility and thus stability throughout development.
Results:
Glycine mutants, which reduced survival at different developmental stages, disrupted egg chamber elongation which requires ColIV polymerisation. Moreover, disrupting 7s domain interactions reduced survival exclusively during late development. As a preliminary characterisation of our novel ColIV timer, ColIV turnover during development was measured revealing higher turnover rate during rapid larval growth followed by slower turnover during pupal stages. We also completed a successful pilot Mass spectroscopy experiment, isolating numerous BM components.
Discussion:
Varying lethal phases among the glycine mutant series may suggest mechanistic heterogeneity. Possible ColIV secretion and network formation defects will be further studied, giving new insight into mechanisms of BM disease. Whilst it’s necessary to form more stable BM during late development, 7s crosslinking may be dispensable in the rapidly turned over early larval BM. Preliminary experiments affirm the feasibility of our ColIV timer and Mass spectroscopy approaches.

P5: Defining the Transcriptional Landscape of Developing Human Tendons.

Alina Kurjan[1], Jolet Y. Mimpen[1], Lorenzo Ramos-Mucci[1], Christopher D. Buckley[2], Adam P. Cribbs[1], Mathew J. Baldwin[1], Sarah J.B. Snelling[1].
[1]The Botnar Research Centre, Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK. [2]Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK.

Introduction:
Tendons are complex extracellular matrix (ECM)-rich tissues that are prone to injury, affecting millions of people worldwide and presenting a significant healthcare challenge and socioeconomic burden. Tendons are capable of regenerative healing during development, but this ability is progressively lost with age, and ECM turnover ceases in late adolescence. Understanding the characteristics of a regenerative tendon could provide crucial clues to developing effective therapeutics for tendon injury. In this study, we aim to establish the histologic characteristics and transcriptomic ECM blueprint of human Achilles tendon development.

Materials and Methods:
Human foetal tendons of 11-20 post-conception weeks (PCW) were obtained from the Human Developmental Biology Resource. Snap-frozen 11-13 PCW (n=6), 15-17 PCW (n=6) and 19-0 PCW (n=4) Achilles tendons were subjected to bulk RNA-sequencing. Snap frozen 20 PCW Achilles tendon midbody samples (n=3) were subjected to single-nuclei RNA sequencing. Formalin-fixed paraffin-embedded 13, 15, 19 and 20 PCW Achilles and patellar tendons were stained with H&E and Alcian Blue to visualise the structure and glycosaminoglycan content.

Results:
Comparison of late 1st trimester (11-13 PCW) and early 2nd trimester (15-17 PCW and 19-20 PCW) Achilles tendon bulk transcriptomes shows that foetal tendon development is characterised by changes in the expression of both collagenous and non-collagenous ECM components, including downregulation of COL3A1, COL5A1, COL9A1, FBN2, FBN3 and TNC and upregulation of COL11A1, COL18A1, MATN4, VIT and VWF over time. At 20 PCW, heterogenous cell type clusters are identified, with Hofbauer macrophages, endothelial cells, muscle cells as well as subtypes of fibroblasts expressing a diverse complement of matrisomal genes. Gene regulatory network and RNA Velocity analyses highlight potential developmental lineage trajectories of foetal tendon fibroblasts by revealing master regulators of tendon developmental cell states and the temporal dynamics of ECM gene expression.

Discussion:
Overall, we provide insights into the ECM transcriptomic profiles of developing human Achilles tendons over time and at single-nuclei resolution. This work lays foundation for further research and functional studies validating the regenerative capacity of the foetal tendon ECM niche with the ultimate aim of indentifying pharmacologic and biomaterial-based therapeutics for guiding tendon repair.

P6: Transcriptional elements driving MMP13 in development.

Sara I AlSalhi[1], Mohammad Alhashmi[1], David Young[2], Simon Tew[1], Kazuhiro yamamoto[1] and George Bou-Gharios[1].
[1]Department of Musculoskeletal and Aging Science, Faculty of Health & Life Sciences, University of Liverpool. [2]Skeletal Research Group, Biosciences Institute, Newcastle University.

Introduction:
Matrix metalloproteinase (MMP13) is a primary catabolic factor involved in cartilage degradation through its ability to cleave type II collagen. Transcriptionally, MMP13 is regulated by two main elements; proximal promoter and distal enhancers. The aim of the study is to identify transcriptional elements that regulate the MMP13 gene in development.
Materials and Methods:
Possible enhancers have been determined by using the Encyclopaedia of DNA Elements (ENCODE), based on histone modifications (Limb H3K4ME1 and Limb H3k27AC), Evolutionarily conserved sequences, fibroblast and muscle peaks, ChIP peaks for RUNX2, and also based on published data on vitamin D elements. Each sequence of possible enhancer was ligated to the silent HSP68 proximal promoter in a vector that expresses ß-galactosidase and been tested in transgenic embryos at E15.5 days.
Results:
We have identified several active enhancers in distal and intronic sequences. Expressions in skeletal elements were detected in the 5th Intron, proximal promoter, and the distal enhancers at -10, -19.4, and -21.5kb). In addition, expression was also seen in other cell types such as developing skin, tendon, and fibroblasts in other tissues. In contrast, the sequence overlapping the highest peak of RunX2 at -29kb did not show significant expression.
Discussion:
Our results showed that the MMP13 is regulated at the level of transcription in at least three different regions, some of which coincide with RunX2 peak activity. We are in the process of identifying cell lineage specific expression in order to combat increase of MMP13 in chondrocytes in osteoarthritis and ligament and tendon rupture that may be associated with MMP activities.

P7: Mapping proteolytic events in extracellular matrix proteins using a label-free quantitative proteomics approach.

Daniel R. Martin[1], Rens de Groot[2], Suneel S. Apte[1], Josefin Ahnström[3] and Salvatore Santamaria[3].
[1]Department of Biomedical Engineering, Cleveland Clinic Lerner Research Institute, Cleveland, USA. [2]Institute of Cardiovascular Science, University College London, London, UK. [3]Department of Immunology and Inflammation, Imperial College London, London, UK.

Introduction:
Proteolysis is a major mechanism of post-translational regulation of extracellular matrix (ECM) proteins. Identification of cleavage sites is important to elucidate the substrate specificity of a certain protease; additionally, the resulting neopeptides can be further investigated as matrikines and/or biomarkers of specific diseases. Here, we aimed to map proteolytic events generated by members of the A Disintegrin And Metalloproteinase with ThromboSpondin motifs (ADAMTS) family on versican, biglycan, cartilage oligomeric matrix protein (COMP) and osteopontin (OPN), all being major components of the ECM in a variety of tissues.
Materials and Methods:
Recombinant purified versican, biglycan, COMP and OPN were digested with recombinant full-length ADAMTS1, ADAMTS4, ADAMTS5, ADAMTS7 and ADAMTS8, using catalytically inactive mutant proteases in control digests. Digestions were analysed on SDS-PAGE and immunoblot. Semi-tryptic peptide abundance ratios determined by liquid-chromatography-tandem mass spectrometry (LC-MS/MS) in ADAMTS:control digests were compared to the mean of all identified peptides to obtain a z-score by which outlier peptides were ranked.
Results:
We identified 21 novel cleavage sites in versican by ADAMTS1, ADAMTS4 and ADAMTS5, 43 in biglycan by ADAMTS4 and ADAMTS5, 48 in COMP by ADAMTS1 and ADAMTS4, and 6 in OPN by ADAMTS8. We confirmed that the Glu441-Ala442 bond is a favoured cleavage site by ADAMTS proteases. We found that ADAMTS4 is the most potent COMPase in vitro. Cleavage of COMP at the Ser77-Val78 site by ADAMTS4 was confirmed using a neoepitope antibody.
Discussion:
The ability of ADAMTS1, ADAMTS4, and ADAMTS5 to cleave at multiple sites in versican may explain the relatively mild phenotype exhibited by mouse strains where the versican Glu441-Ala442 bond was mutated to make it uncleavable by ADAMTS proteases compared to that of Adamts knockout strains. The newly identified cleavage of COMP at Ser77-Val78 by ADAMTS4 is relevant to degenerative joint diseases since a COMP peptide generated by cleavage at this site has been previously reported as a sensitive and specific biomarker of osteoarthritis and rheumatoid arthritis.
Digestion of proteins in vitro and application of our z-score approach is potentially widely applicable for mapping protease cleavage sites using label-free proteomics.

P8: RNA binding protein HuR regulate osteoclast induction capacity of MG63 cells.

Shijian Fu[1], David Turner[1], Simon Tew[1].
[1]Faculty of Health and Life sciences, Institute of Life Course and Medical Sciences, Liverpool, United Kingdom.

Introduction:
The RNA binding protein HuR can regulate different biological behaviors by binding to specific RNA targets. Bone density is mainly controlled by bone formation and bone resorption and can be closely regulated by interactions between osteoblasts and osteoclasts. We have in vivo data that shows that conditional knock out HuR in limb bud mesenchymal cells leads to loss of bone density in adult trabecular bone. This project aims to determine the mechanisms underlying this observation. We have employed an in vitro model that modifies HuR expression in osteoblast-like cells to determine whether this affects their cross-talk with osteoclast progenitors.
Materials and Methods:
Conditioned media, generated from the human osteoblastic MG63 cell line, was used to stimulate osteoclast differentiation of RAW264.7, a murine monocyte/macrophage cell line. M-CSF and RANKL stimulation of the RAW264.7 cells was used as positive control. Differentation was determined by counting cells that exhibit an osteoclast morphology whilst expression of osteoclast-related genes were verified by qPCR. To determine the effect of HuR, it was knocked down in MG63 cells using siRNA. Knockdown was confirmed by western blotting. Condition media from MG63 following HuR knockdown was collected and applied to RAW264.7 cells to identify whether it had altered capacity for osteoclast induction.
Results:
We found that MG63 conditioned media could induce RAW264.7 cells osteoclast formation. However osteoclast induced by MG63 condition media was not as effective as the positive control conditions. qPCR analysis identified that MMP-9 was upregulated in the RAW264.7 cells stimulated with the MG63 conditioned medium while other genes examined (TRAP, CTSK, NFATc-1 and c-Fos) showed no statistical change. HuR was successfully knocked down in MG63 cells by siRNA and the conditioned media collected following this had improved osteoclastogenic capacity, eliciting differentiation comparable to that in the positive control.
Discussion:
Conditioned medium from MG63 cells can induce osteoclastogenesis. However, based upon number and shape of osteoclast induced by MG63 condition media as well as the limited change in marker gene expression, it appears that the osteoclasts formed are immature. Knock down of HuR in MG63 cells facilitates the osteoclast induction capacity of the conditioned medium, which is consistent with the effect of mesenchymal HuR loss at reducing bone density which we have observed in our in vivo analysis. Our ongoing hypothesis is that HuR acts within osteoblasts to limit the production of secreted signals that promote bone resorption.

POSTERS:

P9: Human pluripotent stem cell model of multiple epiphyseal dysplasia with MATN3 mutation identifies upregulation of the cholesterol biosynthesis pathway.

Steven Woods[1], Nicola Bates[1], Stuart Cain[1], Paul Humphreys[1], Fabrizio Mancini[1], Antony Adamson[1], Peter Harley[1], Ian Donaldson[1], Geert Mortier[2], Kate Chandler[3], Clair Baldock[1], Jean-Marc Schwartz[1], Susan J Kimber[1].

[1]University of Manchester, Manchester, United Kingdom, [2]Antwerp University Hospital and University of Antwerp, Antwerp, Belgium, [3]Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.

Introduction:

Multiple epiphyseal dysplasia (MED) is a chondrodysplasia characterised by delayed epiphyseal ossification, short stature, and early onset osteoarthritis. MED can be caused by heterozygous mutations in MATN3, which encodes the extracellular matrix protein Matrilin-3. Human pluripotent stem cells (hPSCs) have excellent potential for providing models of human disease. Here we aim to use our in vitro hPSC model of cartilage growth-plate development to better understand MED. We hypothesise that mutations in MATN3 drive both intracellular and extracellular matrix (ECM) phenotypes.

Materials and Methods:

MATN3 mutant hPSCs were generated from either MED patient PBMCs or by CRISPR-Cas9 gene editing. hPSCs were differentiated to chondrocytes via a mesenchymal cell intermediate (iMSC) followed by TGFβ3+BMP2 induced chondrogenic pellet culture for 21 days. Osteogenic differentiation of iMSCs was performed in monolayer using OsteoMax medium (Millipore) for 28 days. Differentiation and cartilage ECM assembly was assessed using flow cytometry, RNA-seq, immunohistochemistry and transmission electron microscopy (TEM).

Results:

hPSC-derived iMSCs displayed both osteogenic and chondrogenic potential. Mineralised osteogenic cultures stained positive for Alizarin Red and expressed the osteogenic marker transcripts; RUNX2, ALPL, SPP1 and BGLAP. hPSC-derived cartilage pellets stained positive for Alcian Blue and the key cartilage markers; Type-II collagen, Aggrecan, Type-x collagen and Matrilin-3. MATN3 transcript was expressed equally from the mutant and w.t. alleles of MED hPSC-cartilage pellets. Matrilin-3 protein, which is usually detected in the ECM, was partially retained within cells of heterozygous mutant hPSC-cartilage pellets, yet there was only a slight indication of ER-stress. RNA-seq comparison of MED patient and unaffected hPSC-cartilage pellets revealed a significant enrichment for ECM and cholesterol biosynthesis pathway genes. These changes were validated in CRISPR-Cas9 mutant hPSC-derived cartilage pellets. Using TEM we showed abnormal ECM assembly, distended ER, and an accumulation of lipid droplets in MATN3 mutant hPSC-derived cartilage pellets.

Discussion:

Here we have developed a hPSC-derived chondrogenic differentiation model, which furthers understanding of human development and disease. Our model reveals that, mutant Matrilin-3 induces upregulation of the cholesterol biosynthesis pathway and causes abnormal ECM assembly. The intracellular and extracellular phenotype both likely impact MED pathogenesis.

P10: Interaction of Wnts and low-density lipoprotein receptor-related protein 1 in cartilage development.

Abdulrahman M E Gremida[1], Anders Jensen[1], Mohammad Alhashmi[1], Emilie H Mogensen[2], Ida B Thøgersen[2], Jan J Enghild[2], George Bou-Gharios[1], and Kazuhiro Yamamoto[1].
[1]The Department of Musculoskeletal and Ageing Science, Institute of Life Course and Medical Sciences, University of Liverpool, United Kingdom. [2]Department of Molecular Biology and Genetics, Aarhus University, 8000 Aarhus, Denmark.

Introduction:
The low-density lipoprotein receptor-related protein 1 (LRP1) is a 600 kDa cell-surface receptor, which consists of four extracellular ligand binding domains (α-chain) and an 85 kDa transmembrane domain (β-chain). LRP1 is ubiquitously expressed in various tissues and plays tissue-specific roles by mediating the endocytosis of a diverse range of extracellular molecules. Our recent study using limb bud-specific LRP1 knockout mice demonstrated that LRP1 plays an essential role in skeletal formation. Furthermore, our recent unbiased approach to identify LRP1 ligands in cartilage revealed a possible interaction of LRP1 with key components of the non-canonical WNT signalling pathway, namely Wnt5a and Wnt11. Wnt signalling is an evolutionarily conserved developmental pathway that is critical in cell fate determination, organ development and adult tissue homeostasis. In this study, we aim to characterise LRP1 and Wnt interaction.
Materials and Methods:
We validated the interaction of LRP1 and Wnt5a/11 by direct binding assays using purified LRP1 and Wnts. Immunohistochemistry was performed to investigate the regulation of Wnt by LRP1 using LRP1 knockout mice. LRP1-mediated cellular trafficking of Wnts were further investigated using LRP1-deficient fibroblasts.
Results:
We found that Wnt5a and Wnt11 but not Wnt3a, bind directly to immobilised full-length (FL)-LRP1 with high-affinity (apparent binding constant, KD,app, values of 44 and 51 nM, respectively). Our data also shows that one of the four ligand binding domains of LRP1 (Cluster II), which consists of complement-type and EGF repeats without a beta-propeller domain, also binds to Wnt5a and Wnt11. Finally, a conditional LRP1 KO mouse model demonstrated a critical role of LRP1 in regulating Wnt5a and Wnt11 in cartilage in vivo.
Discussion:
These results suggest that Wnt binding domains of LRP1 are different from LRP5/6, which interact with many Wnts but not Wnt5a or Wnt11 via the beta-propeller domain and EGF repeat pairs. This unique interaction of LRP1 and Wnt5a/11 may play a crucial role in the development of cartilage and bone.

P11: MicroRNAs expression between normal and ruptured canine cruciate ligaments.

Yalda Ashraf Kharaz[1], Kasia Whysall[2], Thomas Maddox[1,3], Mandy Peffers[1] and Eithne Comerford[1,3].
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Introduction:
Ligament injury results in severe physical, social, economic consequences to the affected individual and may lead to development of degenerative joint disease such as knee osteoarthritis. Injuries to canine cranial cruciate ligament (CCL) are similar to anterior cruciate ligament injuries in man and are also predisposed to traumatic and non-contact ligament injury. To date, there are no current treatment options targets the prevention of ligament degradation and eventual rupture. MicroRNAs (miRs) control the expression of many genes simultaneously and have been suggested as potential therapeutics for various disorders of the musculoskeletal tissues. To date, there is little known on the role of miRs and its expression in cruciate ligaments during Injury, which could provide targets for alternative medical management CCL injury. This study aimed to investigate the expression of miRs between disease-free CCLs and dogs with CCL rupture.
Materials and Methods:
Disease-free CCLs were harvested from cadaveric canine knee joints (n=4). Ruptured CCLs (n=4) were collected canine stifle joint undergoing routine CCL surgery. RNA was extracted from both groups and qPCR was performed on miRs that have previously been found to be altered in ligament and tendon injury. Predicted target genes for miRs that were found to be differently expressed were produced with TargetScan. Data was analysed using Graphpad Prism and differences between groups were tested using a t-test (α=0.05).
Results:
Amongst miR-29a, miR-26a, miR-146a, miR-146b, let-7f, miR-133, miR-129a significantly higher expression was found in miR-29a (p= 0.039), miR-26a (p=0.05), miR-133 (p=0.001) and miR-129a (p= 0.05) in ruptured CCLs in comparison to disease-free CCLs. Predicated putative target genes for the differently expressed miRs were important extracellular matrix (ECM) genes such as collagen type I alpha-1 chain (COL1A1), collagen type V alpha-1 chain (COL5A1), tenomodulin (TNMD), and Fibrillin-1 (FBN1). In addition putative target genes associated with matrix breakdown such as a disintegrin and metalloproteinase with thrombospondin motifs 5, 7 and 9 (ADAMTS5, ADAMTS7, ADAMTS9) were also identified.
Discussion:
Results indicate that miR-29a, miR-26a, miR-133 and miR129a may be important regulators of CCL ECM during injury and could be used as potential targets to prevent CCL disease and for future development therapies for management of ligament rupture.

P12: LaNt α31 induces an epithelial to mesenchymal-like transition in pancreatic adenocarcinoma cells and changes laminin α3 expression.

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Introduction:
Pancreatic adenocarcinoma is difficult to detect until advanced stages and has poor prognosis. Identifying molecular mechanisms involved in tumour progression is essential to developing new treatments. The laminin protein family has multifaceted roles in regulating of cell behaviours. Expression levels of one member of this family, laminin α3 (LAMA3), predicts patient outcome in pancreatic adenocarcinoma. However, the LAMA3 gene produces three structural and functionally distinct isoforms; LAMA3A, LAMA3B and LAMA3LN1 (LaNt α31 protein). The relative contribution of the different isoforms has not been established and, of particular note, LaNt α31 has never been investigated in pancreatic cancer.
Materials and Methods:
Relative transcript abundance for LAMA3A, LAMA3B and LAMA3LN was compared between four pancreatic cancer cell lines (Mia-PACA-2, Panc-1, Suit-2, BxPc-3). Lentiviral particles were used to generate stable cell line expressing LaNt α31 with photoactivatable-mCherry tag. Expression and its impact upon LAMA3A, LAMA3B, and epithelial-mesenchymal transition (EMT) markers was assessed by western blotting and RT-qPCR RT-qPCR and western blotting. Cell morphology and proliferation rates were measured using an Incucyte system.
Results:
All four tested pancreatic cancer cell lines expressed LAMA3A, LAMA3B and LAMA3LN1 and LaNt α31 at the protein level. However, Mia-PACA-2 cells displayed the lowest expression of all three isoforms and therefore represented a cell line where LaNt α31 effects could be studied largely independent of LAMA3A and LAMA3B.
Overexpression of LaNt α31 in Mia-PACA-2 cells changed cell morphology from cell clumps to spread and individual, suggestive of EMT. Mia-LaNt cells also displayed increased cell-to-matrix adhesion strength. Proliferation assay showed that Mia-LaNt transduced cells grow slower than WT. Mia-LaNt also displayed increased LAMA3B and decreased LAMA3A mRNA levels.
Discussion:
These findings demonstrate that LaNt α31 affects pancreatic cell behaviour and raises the possibility that the observed LAMA3 association with patient outcomes may reflect a hithero unknown contribution from this short splice isoform, possibly to EMT. Moreover, changes to transcripts level of LAMA3 gene could be indicative of feedback switch mechanism regulating LAMA3. Understanding the role of this new protein, the gene switch mechanism controlling it, and investigating the effects of LaNt α31 on cancer progression may help to lead to new treatments.

P13: Therapeutic x-ray doses induce profound structural remodelling in breast extracellular matrix proteins.

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Introduction:
Radiotherapy is widely used as adjunct therapy for breast cancer treatment, thereby exposing normal breast tissue to radiation-associated effects, including fibrosis and increased risk of secondary cancers. Whilst the impact of ionizing radiation (including X-rays) on cellular physiology has been extensively studied, the radiobiology of breast extracellular matrix (ECM) proteins is not well-characterised. Using protein gel electrophoresis and mass spectrometry this study aimed to characterise the differential effects of therapeutic X-ray exposure on the structures of two key breast ECM proteins, collagen I and fibronectin, in purified samples and in a complex matrisome derived from cultured immortalised human mammary fibroblast (HMFU).

Materials and methods:
Purified human collagen I, recombinant cellular fibronectin, and cell-free HMFU matrix were exposed to 300 keV X-rays up to 50 Gy (equivalent to standard breast radiotherapy regimens) in five replicates. The impact of irradiation on protein structure was characterised by native and SDS-PAGE (for collagen I and fibronectin) and by comparing regional differences in peptide yields measured along the protein's primary structure using liquid chromatography-tandem mass spectrometry (LC-MS/MS) following trypsin digestion (peptide location fingerprinting: PLF).

Results:
Exposure to therapeutic X-ray doses induced fragmentation of collagen I alpha chains and fibronectin when visualised by SDS-PAGE. However, the collagen triple helix was unaffected by x-ray exposure on native PAGE. For PLF analysis, MS1 ion intensities from LC-MS/MS were mapped onto the 1° structure. Reproducible and statistically significant (but distinct) changes in peptide fingerprints (PLF) were identified in both purified and complex matrix-derived collagen and fibronectin.

Conclusion:
Our results show that therapeutic levels of ionising radiation likely affects the structure of collagen I and fibronectin. PLF results indicate possible variation in the outcome of radiation damage on ECM components depending on protein source, suggesting a possibility of interplay between different ECM molecules that may induce radiation sensitisation or protection in specific regions of proteins. Further PLF analysis on LC-MS/MS data from the complex HMFU matrisome may reveal other ECM components with significantly altered peptide fingerprints that could, hence, be potential targets for radiotherapeutic studies.

P14: Targeted inhibition of aggrecanases inhibits the progression of age-dependent cartilage degradation in a temporomandibular joint in the STR/Ort mice.

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Introduction:

Temporomandibular joint (TMJ) is one of the common sites developing osteoarthritis (OA). TMJ OA is observed in 8 to 16% of the population. Major symptoms are joint pain, noise, and restricted opening due to structural changes in TMJ, but its pathogenesis remains elusive. In knee OA, cartilage degradation is mediated by collagenases and aggrecanases. We have previously shown that selective inhibition of aggrecanases by a variant of tissue inhibitor of metalloproteinase 3 having an extra alanine at N-terminus ([-1A] TIMP-3) protects against cartilage degradation in mouse OA models. However, little is known about involvement of aggrecanases in the development TMJ OA. In this study, we investigated effect of selective inhibition of aggrecanases by overexpressing [-1A] TIMP-3 in STR/Ort mice, which exhibit human OA characteristics and we have recently shown to develop spontaneous TMJ OA.

Materials and Methods:

We generated transgenic mice overexpressing [-1A] TIMP-3 transgene by infecting fertilized embryo of mice with lentiviral particles (EF-1α [-1A]-TIMP-3). Micro computed tomography (CT) imaging and histopathological examinations were performed to assess spontaneous TMJ OA at 10, 20 and 40 weeks, compared between STR/Ort mice and [-1A] TIMP-3 transgenic mice. These were classified using our original morphological and histological TMJ OA damage scoring systems.

Results:

At 10 weeks in STR/Ort mice, no morphological changes in TMJ were seen. In 20 weeks, bone erosion and osteophyte formation in mandibular condyle were observed. At 40 weeks, the deformity became larger and remarkable bone resorption was seen in some STR/Ort mice. In contrast, [-1A] TIMP-3 STR/Ort mice showed some erosion and osteophyte, but bone resorption was rarely observed. Furthermore, significantly less cartilage degradation in mandibular condylar was observed in [-1A] TIMP-3 STR/Ort mice, compared with control mice.

Discussion:

The TMJ is composed of fibrocartilage containing predominantly collagen I and different from articular hyaline cartilage, which only contains collagen II. However, our data indicates that aggrecanases play a major role in tissue destruction in TMJ OA, suggesting a similar pathogenesis of TMJ and knee OA. Selective inhibition of aggrecanase activity could be a strong approach for treatment of OA in multiple joints.

P15: Investigating the molecular crosstalk mechanisms that drive pathogenesis in idiopathic pulmonary fibrosis.

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Introduction:
Idiopathic pulmonary lung fibrosis (IPF) is a chronic, progressive disease of unknown cause with high morbidity. Transforming growth factor-β (TGF-β) and integrin αVβ6 are key drivers of IPF, inducing epithelial-to-mesenchymal transition (EMT), myofibroblast activation and pathological matrix remodelling. Binding and subsequent force application of integrin αVβ6 to latent TGF-β results in activation and release of TGF-β which in turn promotes IPF. We have identified enrichment of two molecules highly involved in fibrosis, MUC-1 and Galectin-3, in αVβ6 mediated adhesion complexes. MUC-1 regulates mechanical activation of integrins independent of actomyosin contractility. We hypothesise that that MUC1-Galectin-3 interaction regulates αVβ6 driven TGF-β activation to promote IPF progression.
Materials and Methods:
Lung epithelial cells were treated with Gal-3C, Galectin-3, a-αVβ6 (10μg/ml), GO-201 (5μM) or TGF-β (10ng/ml) for up to 24h. Protein levels were measured by immunoblotting. Traction Force Microscopy was used to measure force application in the presence or absence of galectin3-modualting reagents.
Results:
Treatment of cells with Galectin-3 induced β6 protein expression, induction of SMAD2/3 phosphorylation and therefore TGF-β activation. MUC-1 inhibition with GO-201, a peptide blocking the MUC-1 oligomerisation, suppressed of αVβ6, but had no effect on Galectin-3 protein levels. Whereas, treatment with TGF-β increased both αVβ6 and Galectin-3. Gal-3C, a truncated peptide inhibiting Galectin-3 binding partner oligomerisation, increased mechanical force application, suggesting that Galectin-3 oligomerisation reduces the forces exerted by the cells on fibronectin.
Discussion:
These data reveal a positive regulatory feedback mechanisms between αVβ6 and TGF-β but also TGF-β and Galectin-3. Both Galectin-3 and MUC-1 regulate αVβ6 directly or indirectly and Galectin-3 regulates cellular mechanics. As MUC-1 oligomerisation promotes integrin clustering, future studies will examine whether MUC-1 oligomerisation alters αVβ6 localisation, Galectin-3-dependent αVβ6-mediated mechanoactivation of TGF-β.

P16: Increased LaNtα31 activates Akt signalling in breast cancer and changes the mode of tumour invasion into laminin matrixes.

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Introduction:
Invasion and metastasis of tumour cells are complex processes involving the interaction of cancer cells with laminins and collagens in the extracellular matrix. Laminin N-terminus α31 is the newest member of laminin family, derived by alternative splicing from the LAMA3 gene. Recently, LaNtα31 has been demonstrated to be upregulated in breast cancer, with further upregulation in distal metastases.
Materials and Methods:
To determine the impact on cancer cell behaviour, LaNtα31 was upregulated using adenoviral mediated transduction in invasive and non-invasive breast cancer cell lines and spreading, migration and invasion assays. Effects of LaNtα31 on activity of the FAK, SRC, PI3K/Akt and P38MAPK, key cell signalling cascades of matrix remodelling and regulation of epithelial to mesenchymal or amoeboid transitions, were assessed by Western blot analysis.
Results:
Strikingly, LaNt α31 overexpression changed the mode of MDA-MB-231 breast cancer cell invasion from multicellular streaming to individual cellular invasion. However, this effect was only observed when invading into a laminin-containing matrix. Overexpression of LaNtα31 led to a sustained increased Akt phosphorylation, with less dramatic changes observed in the other signalling pathways.
Discussion:
These results suggest that the LaNtα31-induced changes in cell invasive behaviour are driven by activation of Akt, a major player in driving epithelial to mesenchymal transition. These findings have broad implications in the understanding of LaNtα31 and laminin regulation of tumour progression.

P17: Substrate stiffness does not affect bone marrow stromal cell differentiation: the importance of cell density and substrate thickness.

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Introduction:
The ECM elastic modulus affects cell behaviour. However, the stiffness that cells sense is determined not only by substrate modulus but also by geometry, such as substrate/matrix thickness and cell crowding. In this study, we hypothesised that these factors would modulate cell-traction-induced matrix deformations and modulate cellular differentiation.
Materials and Methods:
'Soft' and 'stiff' polyacrylamide (PAAm) hydrogels of different thicknesses adhered to an underlying glass support were characterised by nanoindentation and confocal microscopy. BMSCs cell spreading and actin fibres formation were measured using microscopy. Proliferation was measured using a PicoGreen assay, and osteogenic and adipogenic differentiation were measured by qPCR and histology. Cell-induced tractions were measured by displacement microscopy of gels embedded with fluorescence fiduciary beads at different seeding densities on soft, thin and thick PAAm hydrogels in basal, osteogenic and adipogenic media.
Results:
Hydrogel stiffness was significantly greater for thin vs. thick materials despite identical gel composition. BMSC spreading was lower on soft, thick gels compared to soft, thin gels. No significant differences in osteogenic and adipogenic differentiation were measured relative to gel elasticity and thickness. Stro-1+ BMSCs induced significantly greater deformations in soft, thick compared to soft, thin PAAm hydrogels. These deformations decreased significantly with respect to growth time and cell density. Cell deformations were significantly greater in cells cultured in osteogenic compared to basal conditions.
Discussion:
The observation that individual cells spread to a greater extent on thick compared to thin hydrogels of identical composition illustrates that shear cell tractions are resisted by the underlying glass support. Similar resistance to nanoindentation likely explains the higher stiffnesses measured using this technique. However, the finding that displacements decreased at high cell densities suggests that cell density also constrains cell tractions, and may explain the lack of difference in osteogenic differentiation as a function of gel stiffness.

P18: The cell-surface receptor LRP1 is a rapid responder and transducer of mechanical force.

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Introduction:
The low-density lipoprotein receptor-related protein 1 (LRP1) is a ubiquitously expressed cell surface receptor and regulates tissue remodeling, inflammation, and extracellular molecules through endocytosis. It plays a crucial role in cartilage tissue homeostasis but little is known about its role in mechano-transduction. We hypothesis that LRP1 is a rapid responder during injury to maintain tissue stability.
Materials and Methods:
Mouse embryonic fibroblasts (MEF's) wild-type (WT) and LRP1 knockouts (KO) pellets were subjected to mechanical insults of 283 kPa and 400 kPa in a high and low ligand environment. A mechano-culture system was designed to pressurize a culturing vessel reaching a maximum of 283 kPa and 400 kPa within 2 seconds, with a single cycle stimulus taking 32 seconds to release the air entrapped within. The level of LRP1 and nuclear intermediate filament, Lamin AC, were examined using Western blot analysis. Lamin AC was used as a measure of force response / sensing, it has been shown to adjust within seconds in response to force, maintaining the cells nuclear structural stability under applied force.
Results:
LRP1 protein levels in MEF's WT increased 20 folds at 283 kPa force at 10 cycles of stimulation, but no significant increase was seen at 400 kPa of force. At 283 kPa of force, LRP1 increased significantly in a high ligand environment but not in a low ligand environment. The WT cells showed a 7-fold lower Lamin AC levels compared to the KO cells without stimulation. Furthermore, at 283kPa of force in a ligand rich environment, the WT cells significantly increased Lamin AC levels, whereas no significant change of the Lamin AC levels was observed in the KO cells. When WT and KO cells were subjected to 283 kPa mechanical force in a low ligand environment, no significant Lamin AC differences were seen between them in response to force.
Discussion:
These data shows that LRP1 has a mechanical threshold response. In a ligand free environment, the nuclear mechanical response of the WT and KO cells are similar, signifying LRP1 ligand interaction regulates nuclear mechanics. This study suggests that LRP1 is a rapid mechano-responder in cells that are exposed to acute injury.

P19: Optimisation of Umbilical Artery Endothelial Cells Denudation for Endothelial-Vascular Smooth Muscle Cells Signalling Ex Vivo.

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Introduction:
Endothelial-vascular smooth muscle cells (EC-VSMC) interaction is fundamental to the function of the vasculature. In the event of endothelial injury, the smooth muscle cells are activated and proliferate towards the intima before re-endothelialisation occur. This is known as intimal hyperplasia (IH) where the blood vessel's intima layer is thickening. Subsequent event following endothelial injury that leads to IH development are yet to be elucidated. Therefore, re-endothelialisation mechanism has become priority to be elucidated especially in ex vivo setting which mimic to the actual event in order to combat IH.

Materials and Methods:
In this study, healthy human umbilical arteries (hUAs) have been utilised and was denuded from endothelial cell to set up an ex-vivo model for EC-VSMC interaction study. Therefore, we aimed to optimise the hUA denudation method prior to establish an ex-vivo IH model. The denudation of human umbilical artery was assessed by comparing two types of dissociation enzymes; trypLE Select and trypsin-EDTA at different incubation time. Immunohistochemistry was performed to determine the degree of denudation via quantifying endothelial cell coverage post-denudation protocol.

Results:
Result shows that denudation protocol using trypLE Select and trypsin-EDTA at 5–10 minutes removed more than 80% of the endothelial cells whilst 3 minutes incubation period removed 50% of the endothelial cell.

Discussion:
Both trypLE select and trypsin-EDTA are able to gently remove majority of endothelial cells on the lumen. This set up model will enable us to understand the re-endothelialisation event and EC-VSMC interaction when the endothelial-like cells are seeded on the denuded artery thus, subsequently increase the patency of the vessel graft.

P20: Characterization Of The Biophysical Properties And Cell Adhesion Interactions Of Marine Invertebrate Collagen From Rhizostoma pulmo.

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Introduction:
Collagen is the most ubiquitous biomacromolecule found in the animal kingdom and is commonly used as a biomaterial in regenerative medicine therapies and biomedical research. The potential of the mammalian collagens used in these applications is limited by widespread religious constraints and the continuous risk of zoonotic disease transmission. These issues have led to increasing research into alternative collagen sources, of which marine invertebrate collagens have emerged as a promising resource. The Mediterranean scyphozoan Rhizostoma pulmo presents an attractive alternative collagen source; however, there is little data surrounding the biochemical characterization of the collagens from R. pulmo (JCol). Here we evaluate some of the key biochemecial properties and cell adhesion mechanisms of JCol.
Materials and Methods:
Biochemical characterization of JCol included SDS-PAGE, circular dichroism spectroscopy, heparin-affinity chromatography, atomic force microscopy (AFM) and a fibrillogenesis assay. The 2D interactions of human fibroblasts, fibrosarcoma and mesenchymal stem cells with JCol were evaluated using adhesion and proliferation assays, as well as an integrin binding inhibitory antibody assay.
Results:
JCol was shown through SDS-PAGE to contain a heterogenous mixture of glycosylated proteins, of which several correspond to equivalent molecular weights of α1 and α2 collagen chains on Type I collagen from rat tail. The presence of collagenous proteins was confirmed through the observation of a typical triple-helical molar ellipticity spectra, and through the observation of fibril formation under AFM. In-vitro cell assays on JCol substrates demonstrated the absence of α2β1 integrin binding motifs as well as a significantly greater proportion of heparan-sulfate chain binding compared to rat tail collagen. The presence of heparan-sulfate binding motifs on JCol was confirmed through heparin-affinity chromatography.
Discussion:
These findings report the first images of collagen fibres derived from R. pulmo as well as a background characterization of its biophysical properties, fibrillogenesis dynamics and cell adhesion interactions. The absence of α2β1 integrin binding motifs on JCol and greater proportion of heparan-sulfate chain binding suggests that this marine invertebrate collagen posseses significant differences in amino acid sequence to mammalian fibrillar collagens and must be further evaluated to deterermine their suitablilty for biomedical applications. This work provides the initial framework for future biomedical research which may benefit from atypical, non-mammalian collagen-cell interactions.

P21: Developmentally Inspired Model of Endochondral Ossification.

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Introduction:
In postnatal development, chondro-osseous transitions such as endochondral ossification (EO) are regulated by rapid matrix turnover, mineralisation and chondro-osseous transdifferentiation, all disrupted in osteoarthritis (OA). We're exploring how force governs these transitions of cartilage to bone; previous studies from our group indicate cartilage matrix and chondrocyte phenotypic plasticity in the growth plate, and stability in articular cartilage, is mechanoregulated. Here we describe exploition of a human 'developmental biology-inspired platform' alongside in vivo studies, to study cartilage mineralization.
Materials and Methods:
In vivo studies reveal chondro-osseous transitions are inhibited by forces. We synthesise GelMA using click chemistry to generate hydrogels with compressive moduli of 3-5 kPa. Bouyancy-driven gradients of BMP-2 within hydrogels seeded with hMSCs were cultured for 28 days. qPCR, histology and immunohistochemistry assessed, cartilage, hypertrophic and bone markers. Uniaxial cyclic compression (0.5Hz, 10% strain) was applied using Electroforce5500. Cells from Confetti-UBCre mice are being used for lineage tracing studies.
Results:
MSC-laden GelMA constructs showed differential gene expression across the gradient, indicating tri-phasic osteochondral tissue formation within 28 days. Runx2/Sox9 immunostaining confirmed osteochondral differentiation, increased expression of SPP1, SP7, Runx2, Col1 indicated osteogenesis at one end, while distinct expression of Col10 and Runx2 in the central region marked cellular hypertrophy. The effects of cyclic loading on cell signalling, hyaline cartilage formation and thickness, calcification and phenotypic plasticity/stability are being assessed and compared to in vivo findings.
Discussion:
These data validate the formation of a humanised osteochondral gradient recapitulating developmental processes, in vitro. It is hypothesized that the generated osteochondral tissues will reflect the results of phenotypic changes in cells and ECM regulation, under physiological and pathological loads. The model will be integrated with snRNA sequencing and lineage tracing, to study trans-differentiation. This approach provides an experimentally tractable mechanobiology model and clinically conformant osteochondral tissue development model enabling fundamental biology and disease modelling across scales.

P22: A computational platform to study the role of extracellular matrix mechanics in tissue development and homeostasis.

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Introduction:
The extracellular matrix (ECM) is a non-living complex protein meshwork that surrounds many tissues, acting as a physical scaffold for the living cells to adhere to, as well as providing a platform for biochemical cues. A plethora of literature have studied the biochemical/mechanical signalling pathways downstream of ECM, often treating it as a static platform. Recent studies are shedding light on the pure mechanical role of ECM in sculpting tissues. Furthermore, the interactions between ECM and its overlaying living cells can give rise to dynamic mechanical properties, which can then feed back to affect cell behaviour and tissue shape. However, characterising these dynamic mechanical properties and their effect on tissue shape is experimentally challenging. We propose a computational platform to study the role of ECM mechanics in shaping tissues.

Materials and Methods:
The computational platform is a generalisation of a Finite Element Model previously developed to study folding of the Drosophila wing disc. The platform assumes a Neo-Hookean material model and takes tissue geometry, growth rate and mechanics as input. These can be directly calculated from experimental measurements or defined as a range for hypothesis testing. The platform comes with a graphic user interface for better visualisation and ease of use.

Results:
Our generalised computational platform can incorporate different starting geometries, boundary conditions and material properties. In particular, the input material properties can be controlled both spatially and temporally, allowing the user to test the effect of different heterogeneities on tissue shape. The platform also includes different folding mechanisms and mechanical tests. Together, these make the platform applicable to a variety of physiological tissues and multicellular in-vitro systems such as organoids.

Discussion:
Spatiotemporal changes in cell and ECM properties can affect tissue shape. Our computational platform can be used as a hypothesis-testing tool to study the effect of these changes. The platform can also be used to simulate growth of organoids in matrices with different mechanical properties, which can then guide tissue engineers to tailor-design ECM platforms for organoid growth.

P23: Decellularised Human Saphenous Vein Graft in Porcine Carotid Artery Model: Tissue Remodeling & Cell Infiltration Analysis.

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Introduction:
Saphenous vein graft (SVG) are the most use graft in Coronary artery bypass grafting (CABG). Unfortunately, SVG has a low long-term patency with graft failure rate of 40−50% 10 years after bypass. The prevalence of SVG graft failure may be due to the anatomical and physiological properties of the conduit that are dissimilar to the coronary position it is grafted onto i.e. vein tissue into arterial position. Cells populating both veins and arteries themselves are genetically pre-determined, thus were postulated to contribute in vein graft adaptation in the arterial position. By arterializing the veins, the graft patency consequently could be increased. Arterialisation are done by decellularising the veins leaving only the extracellular matrix (ECM) as a bare natural scaffold for repopulation by host cells thus producing an autologous graft.

Materials and Methods:
SVG were decellularised as previously described and then implanted in female Landrace pigs (n=3). Implanted grafts were surgically exposed and evaluated after 4 weeks via Doppler ultrasound and histological staining. Sections were stained with H&E, Picrosirius Red, EVG and Alcian blue to visualised conduit lumen diameter, wall thickness, and extracellular matrix composition. Collagen, elastin and glycosaminoglycan (GAGs) were also assayed to evaluate the matrices pre-and post-implant. Cells repopulating the decellularised graft were also analysed with markers of endothelial cells (EC), smooth muscle cells (SMC), Monocytes (Mo)/macrophages (Mϕ) and fibroblast cells.

Results:
Decellularised SVG showed good mechanical strength and biocompatibility without mechanical failure, and 2/3 patency rate at 4-weeks. All 3 implants outcome varies with one of the implant were not patent due to atherosclerosis, whereas the other 2 samples were patent after 4 weeks but each shows either outward or inward remodeling. EC, SMC and Mo/Mϕ were observed infiltrating the decellularised SVG with no fibroblastic cells detected.

Discussion:
Our findings demonstrate that decellularised SVG were repopulated with host cells after 4 weeks in vivo and support preliminary feasibility testing in porcine carotid artery. Long-term in vivo data is needed to ascertain the role of the proposed approach a pre-clinical graft testing model and in the clinical setting.

P24: Developing new imaging tools to understand laminin network formation and Remodelling.

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Introduction:
The laminin family are extracellular matrix proteins that play multiple important roles including: biomechanical support of sheets of cells, regulation of cell migration, defining differentiation, proliferation and signalling pathway activity and as part of barriers to tumour invasion. Despite this importance, there remain key unanswered questions, particularly with regards to laminin secretion, the self-assembly dynamics of laminin network assembly, and remodelling. This area is of high interest as recent work has established that proteins that influence laminin interactions, including netrin-4 and laminin N-terminus protein (LaNt) α31, have potent effects on normal tissue and tumour behaviour. In part, the knowledge gaps exist due to a lack of appropriate tools to address these questions. The objective of this project was to develop a suite of new tools, with super-resolution capability, to allow the study of laminin dynamics, interactions and turnover which has never before been possible.

Materials and Methods:
New laminin-β3 and LaNt α31 expression tools were generated with coloured conventional and/or photoactivatable (PA) fluorescent proteins by synthesising genetic fragments, including the full-length human forms of these proteins and cloning them into expression constructs. Fluorescent tags were sub-cloned on the amino- and carboxy- terminal ends by restriction enzyme digest. The plasmids were transfected into human embryonic kidney cells or corneal epithelial cells. Proteins were extracted for processing by western blot with antibodies against mCherry and GFP. Cells were either imaged live by spinning disk, TIRF, and lattice-SIM fluorescence microscopy, or fixed and processed with antibodies against laminin-α3 and laminin-γ2 chains.

Results:
Western blotting confirmed expression of the predicted sized proteins. Transfected corneal epithelial cells showed deposition of pCAG-PA-mCherry-LAMB3-GFP outside the cells in the archetypal laminin deposition pattern and co-localisation with laminin-α3 and laminin-γ2, indicative of LM332 heterotrimer formation. Super-resolution microscopy confirmed successful activation of PA-mCherry and PA-GFP double-tagged laminin-β3 and photoactivation of LaNt α31-PA-mCherry using TIRF in live corneal epithelial cells.

Discussion:
The laminin-β3 double- and single-tagged constructs and LaNt α31-PA-mCherry are exciting new super-resolution tools for understanding laminin network formation and remodelling. Next steps include lentiviral packaging for stable expression, dynamics experiments observing deposition, spatially resolving the amino- and carboxy-end PA-tags and LaNt α31-PAmCherry co-localisation studies.

P25: Optimisation of a proteomic workflow for the extraction of extracellular matrix proteins within dental tissues.

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Introduction:
Dental diseases, such as periodontal disease are prevalent worldwide and are more frequent with age, commonly leading to tooth loss when untreated. Additionally, dental diseases such as equine odontoclastic and tooth resorption hypercementosis are also important age-related equine dental diseases. The underlying reason for dental disease across all species is poorly understood. Principal dental tissues include enamel, dentin, cementum and pulp. Extracellular matrix (ECM) protein initiates crucial biochemical and biomechanical cues for various biological processes. However, due to the low abundance and inaccessibility of ECM proteins in teeth, the protein extraction techniques often involve pooling multiple samples together. Thus, most research has not examined multiple tissues from a single tooth but opted for combining separate teeth with the aim of identifying more proteins. In this study, we aim to identify age and disease specific protein profiles in different dental tissues.
Materials and Methods:
Bovine teeth were collected and dentin and enamel were isolated by removing the pulp with endodontic files and any adhering soft tissue with a scalpel. Hard tissue was crushed into a powder and proteins were extracted in a sequential process with the chelating agent, ethylendiaminetetraacetic acid (EDTA) and the chaotropic detergent, guanidine hydrochloride (GnHCl). In-solution trypsin digestion, followed by mass spectrometry on a QEx-active was undertaken and protein profiles determined following identification using MASCOT.
Results:
We have isolated dentin and enamel from bovine incisors using an IsoMet low speed precision saw and diamond wire. ECM proteins were successfully extracted from these tissues and prepared for mass spectrometry-based protein identification. We identified a total of 91 proteins and all MASCOT hits were cross referenced against previous proteomic data and enamel and dentin specific proteins were detected.
Discussion:
These preliminary data show that dental tissues from single tooth can be isolated and analysed using our proteomic approach. Future work will involve the analysis of human and equine clinical samples using a similar approach to compare not only individual tissues but also the relationship between each tissue in a single tooth. Our approach for dental proteomics aims to identify certain ECM proteins, which are important in ageing and disease.

P26: Dynamic compression modulates chondrogenesis of ATDC5 cells in an agar hydrogel.

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Introduction:
Hydrogels are a promising tool in the effort to engineer biomimetic tissue for in vitro studies of articular cartilage. Mechanical stimulation has been shown to improve chondrogenesis in hydrogel encapsulated cells. Our previous studies have demonstrated that a sinusoidal compression regime of 0.33Hz at a force of 10kPa led to an increase in proteoglycan (PG) content and upregulation of chondrogenic markers (Col2a1, Sox9) in agar encapsulated ATDC5 cells. This regime closely mimics the physiological gait of a walking human at a slower pace. In this study, we aim to further this by testing other compression regimes and gaits to mimic trauma and assess the effect of dynamic compression on cell viability and ECM production.

Materials and Methods:
106 ATDC5 cells/ml were encapsulated in 2% v/w agar. Hydrogels were compressed at day 7 with 4 (sinusoid, triangle, square, sawtooth) compression regimes. The sinusoid compression samples were compressed for a further 14 days at 1Hz. TUNEL assay and qPCR were used to quantify cell apoptosis and gene expression (Col2a1, Col1a1, Sox9) respectively. Scanning electron microscopy and picrosirius red staining were used to assess ECM deposition.

Results:
Col1a1 was significantly upregulated following triangle compression, while square and sawtooth significantly downregulated Col2a1 expression. Cell death was significantly increased in all regimes, with sinusoidal compression resulting in the lowest and sawtooth the highest death. Cells observed at 21 days showed ECM development with adherence to the hydrogel surface. However, no significant differences were found between compressed and uncompressed samples.

Discussion:
We demonstrate that trauma-like mechanical loading downregulates markers of chondrogenesis (Col2a1) while upregulating dedifferentiation (Col1a1) markers. This may be due to trauma regimes not allowing proper relaxation of the cells between force application. ECM production was observed after 21 days of culture following a 1Hz sinusoidal regime. However, no significant difference between compressed and uncompressed samples indicate that hydrogel encapsulation might be the predominant factor driving ECM deposition. Other studies have highlighted the importance of hydrogel stiffness as a factor involved in cell adhesion and ECM development. We will therefore investigate modulation of the mechanical stiffness of our hydrogels in the future.

P27: Coated Commercially Available Matrix as an Alternative Coronary Graft: Patency Analysis.

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Introduction:
Coronary artery bypass grafting (CABG) requires the use of grafts to revascularised the heart. The most used graft i.e. saphenous veins have a low long-term patency hence the need for an alternative graft is of high interest. The use of synthetic, xeno- and allo-graft has been successful in large diameter vessel but less so in < 5mm vessel. Thus, we hypothesised that chemokine and cytokine coated acellular matrix could drive the graft adaptation into a more autologous graft through repopulation of host cells.
Materials and Methods:
Commercially available matrix was coated with chemokines and cytokines and implanted infemale Landrace pigs (n=12). The animal model was divided into 2 groups, treatment (n=6) and control (n=6). After 4 weeks of implantation, the graft was surgically removed and analysed. The patency of the graft was analyse based on the lumen size pre-implantation, and post-implantation (proximal and distal section as compared to the graft section) via Doppler ultrasound.
Results:
The treatment group showed 50% patency (n=3) and 50% occlusion (n=3) similar with the control group. There were no significant differences in the lumen size pre-implantation versus post implantation in the proximal and distal section in the treatment group. Whereas, control group showed significant differences in the lumen size at the distal section of the occluded grafts post-implantation.
Discussion:
Treatment and control group showed similar patency, yet the lumen size in both groups varies base on lumen size proximal or distal to the graft post-implantation. In all implants, vessel lumen distally to the graft were noted to be smaller in diameter than native carotid (i.e. preimplant). Occluded control graft were noted to have significantly bigger lumen proximal to the graft than occluded treated grafts after being implanted. Thus shows the effect of coatings on graft adaptation post-implantation and possibly estimate patency of the graft after 4 weeks. Further analysis on explanted grafts are needed to ascertain that coatings does helps with graft adaptation post-implantation.

P28: Matrix turnover quantified in mouse tendon by isotope labelled proteomics.

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Introduction:
Heavy carbon isotopes in the tendons of people who grew up in the age of nuclear bomb testing have shown that the extracellular matrix, assembled during development, stays with us for life. However, recent work suggests that type-I collagen, in mouse tendon, exists in two pools: a permanent matrix, and a more soluble, circadian-regulated matrix. Here, we use stable isotope labelling coupled with mass spectrometry to quantify the half-lives of tendon matrix proteins.
Materials and Methods:
Tail tendon was harvested from 22-week old C57/BL6 mice fed on a heavy-lysine (13C-Lys; CK Isotopes) diet for either 1, 2, 4, or 8 weeks (n = 3). Tail tendons from 8-week old mice, fed on the heavy diet for 4 weeks were also collected (n = 3). Protein was extracted from tissues using a sequential two-step protocol: fraction 1 (F1), in sodium laurate/deoxycholate; and fraction 2 (F2), in sodium dodecyl sulphate with ultra-sonication (Covaris). Solubilized fractions were analysed by liquid-chromatography coupled tandem mass spectrometry (Thermo).
Results:
The soluble F1 fraction was found to contain intracellular proteins, and a range of core and associated extracellular matrix proteins, including a circadian-regulated pool of type-I collagen. The F2 fraction contained primarily collagens, including type-I collagen which did not show rhythmicity. Type-I collagen had significantly different half-lives of 4 ± 2 days in F1, compared to 700 ± 100 days in F2. Additionally, matrix proteins extracted in the F1 pool had significantly shorter half-lives than F2. In the younger, 8-week old, mice this separation was lost and there was no longer a significant difference between the turnovers of the two fractions. Overall circadian-regulated matrix proteins were found to have significantly faster turnover. More generally, matrix protein half-lives could be placed in a hierarchy of collagens > glycoproteins and proteoglycans > matrix-associated proteins.
Discussion:
The soluble F1 fraction was found to contain intracellular proteins, and a range of core and associated extracellular matrix proteins, including a circadian-regulated pool of type-I collagen. The F2 fraction contained primarily collagens, including type-I collagen which did not show rhythmicity. Type-I collagen had significantly different half-lives of 4 ± 2 days in F1, compared to 700 ± 100 days in F2. Additionally, matrix proteins extracted in the F1 pool had significantly shorter half-lives than F2. In the younger, 8-week old, mice this separation was lost and there was no longer a significant difference between the turnovers of the two fractions. Overall circadian-regulated matrix proteins were found to have significantly faster turnover. More generally, matrix protein half-lives could be placed in a hierarchy of collagens > glycoproteins and proteoglycans > matrix-associated proteins.

P29: An intron-retention splicing switch provides dual layers of laminin alpha3 regulation.

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Introduction:
The LAMA3 gene generates structurally and functionally distinct laminin proteins; laminin alpha3a (transcript LAMA3A) and laminin alpha3b (LAMA3B). A non-laminin third protein, termed Laminin N terminus alpha31 (LAMA3LN1) is also produced from LAMA3 via intron retention and alternative polyadenylation. This protein is a potent mediator of basement membrane assembly and function, likely via impacting upon laminin network assembly. LAMA3LN1 and LAMA3B share a promoter, which raised a possibility that the splicing event could represent a previously unknown form of laminin expression regulation.
Materials and Methods:
Gene array and patient outcome data were interrogated to identify relationships between outcomes and expression-levels of the LAMA3 transcripts. RT-qPCR was used to analyse expression differences cultured cells and cells following cytokine treatment, calcium switch or hypoxia. A dual colour splicing minigene with point mutations affecting critical residues was transfected into cells and splicing rates analysed by flow cytometry, western blotting and fluorescence microscopy.
Results:
Array data analyses identified that the LAMA3A to LAMA3B ratio provided better patient outcome prediction than markers for either transcript independently for lung, breast and gastric cancers. However, this ratiometric effect was reduced when a marker within the shared region of LAMA3B and LAMA3LN1 was used. This finding generated a hypothesis where the splicing and polyadenylation rates within LAMA3 are regulated as a mechanism to control the LAMA3B to LAMA3A ratio. LAMA3LN1 expression levels were determined as accounting for 10 to 50% of the LAMA3B promoter-derived products, and the relative ratios changed in response to cellular conditions and cytokines. Minigene experiments validated the RT-qPCR findings and mutational analyses established that donor splice site and acceptor splice site strength are primary determinants of intron retention and alternative polyadenylation rate, with RNA-binding protein sequences at the donor site also contributing at a lower level.
Discussion:
Together these findings identify intron retention and alternative polyadenylation as a hitherto unknown contributor to LAMA3 regulation and, importantly, point to a two-layer regulation system with this splicing switch controlling LAMA3B:LAMA3A ratio while the produced LaNt alpha31 regulates laminins and basement membrane function at the protein level.

P30: Characterising the relationship between muscle wasting and osteoarthritis in post-traumatic and spontaneous models of osteoarthritis.

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Introduction:
Musculoskeletal tissue dysfunction is the leading cause of frailty, falls, and decreased quality of life in older people. Osteoarthritis (OA), the most common chronic joint disorder, was initially characterised by deterioration of the articular cartilage, though it is now considered to be a disease of the entire joint. Linked to the joint, periarticular muscles also play a major role in joint function and stability. Muscle wasting (MW) is defined as the loss of muscle mass and strength with age. Despite the high prevalence of both MW and OA in older populations, little is known about the interaction between the two.
Materials and Methods:
Male outbred CD-1 mice were used as a spontaneous model of OA and were culled at either 3-months (n=9), 4-months (n=5), 6-months (n=10), or 8-months (n=6). For the model of post-traumatic OA, mice were subjected to a two-week regimen of mechanical loading and aged for 12 weeks post-loading (n=22). Gastrocnemius and quadriceps muscle sections were used to determine fiber cross-sectional area (CSA). Joints were scored for OA severity using the OARSI grading system. qRT-PCR was used to quantify miRNA and mRNA expression in the periarticular muscles.
Results:
CD1 mice exhibited significant OA development at 6- and 8-months of age compared to their 3- and 4-months old counterparts ($p<0.01$). Aged CD1 mice also showed significant reduction in CSA in the gastrocnemius ($p=0.003$), and upregulation of atrophy marker 'ATROGIN' ($p<0.05$), indicating MW. In PTOA groups, mice showed a significant reduction in fiber CSA in the quadricep muscles of the limb with PTOA ($p=0.026$), which correlated with OA severity ($p=0.04$). miR-378a-3p, which was previously shown to be decreased in MW, was downregulated in the quadricep muscles of the limb with PTOA ($p=0.039$), but not in spontaneous OA. Moreover, it's autophagy-associated target genes - P62 and NRF1 - were upregulated ($p<0.05$).
Discussion:
This research is the first to characterise muscle wasting in non-surgical murine models of OA and provides evidence that OA and MW are intricately linked. Moreover, we suggest a role of microRNAs in their interaction, in particular miR-378a-3p, whose targets include genes critical to mitochondrial regulation and ECM maintenance.

P31: Circadian clocks, extracellular matrix and endochondral ossification in the intervertebral disc.

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Introduction:
The circadian clock is a biological time-keeping mechanism that temporally coordinates physiological and behavioural processes. Chronic disruption to circadian rhythms (through ageing or shift work) is thought to contribute to a multitude of diseases, including degeneration of the musculoskeletal system. The intervertebral disc (IVD) is known to possess an intrinsic circadian clock that drives downstream rhythmic gene transcription. These rhythmic outputs are essential for coping with the daily challenges that the intervertebral disc is exposed to within its unique physiological niche.
Materials and Methods:
Spines from 3, 6 and 12 month old Col2a1 specific Bmal1 KO mice were analysed using RGB staining and IHC for selected genes. Changes in IVD matrix properties were investigated using atomic force and electron microscopy. Changes in global transcription levels between WT and KO mice were assessed with RNA sequencing.
Results:
In this work we characterised the age-related degeneration that occurs within the intervertebral disc of the Col2a1-Bmal1 knockout mouse. A major endochondral ossification-like phenotype in the annulus fibrosus was observed. Circadian time series RNAseq revealed that this phenotype was associated with not only the loss of circadian patterns, but also the emergence of ultradian rhythms in gene expression of the whole IVD. Other aspects of intervertebral disc homeostasis, such as extracellular matrix microarchitecture and mitochondrial homeostasis, were also disrupted within the annulus fibrosus of Bmal1 knockout mice. Further RNA-sequencing identified tissue-type specific transcriptional changes, supporting apparent loss of annulus fibrosus-like phenotype markers in Bmal1 knockout mice.
Discussion:
Collectively, these data highlight the importance of circadian rhythms in the maintenance of the cell fate and extracellular matrix homeostasis of the intervertebral disc. Further studies may identify potential molecular targets for alleviating intervertebral disc degeneration.

P32: Identifying alternative polyadenylation events associated with osteoarthritis.

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Introduction:
Alternative polyadenylation (APA) is a process that can produce multiple transcript isoforms with different length 3'UTR and even coding sequences. This can have significant effects on post-transcriptional regulation of genes as well as, in some cases, affecting protein functionality. Examining global incidence of APA events in osteoarthritis has not been performed and may provide mechanistic understanding of the pathophysiology whilst providing targets for therapeutic development.
Materials and Methods:
Age matched healthy and osteoarthritic knee articular cartilage RNA was sequenced using the Quant-Seq Reverse technique and then reads aligned using STAR. Differential expression was analysed with DeSeq2 and APA events were assessed using expressRNA. APA RNA isoforms that exhibited disease specific changes were further analysed using qPCR assays. In vitro analysis of mRNA half-life and cytokine responsiveness of these APA variants was performed in human articular chondrocytes.
Results:
Osteoarthritis led to widespread changes in overall gene expression levels, as has been shown in previous studies. We found that the effect of osteoarthritis on polyadenylation site usage was relatively modest however. Analysis using expressRNA identified 20 genes with differences between proximal and distal polyadenylation site usage between healthy and osteoarthritic samples. In vitro analysis showed that the genes OSMR and KMT2A exhibited a difference in mRNA stability between APA isoforms. The ratio of OSMR isoforms was also modulated in response to interleukin 1B exposure.
Discussion:
Widespread changes in the pattern of polyadenylation site usage site usage is not a widespread characteristic of osteoarthritis. Nevertheless, disease associated APA is observed in a selection of genes which affects post-transcriptional regulation and can be responsive to inflammatory factors. OSMR in particular is an interesting candidate as APA leads to an intronic polyadenylation event, resulting in he production of a secreted receptor that antagonises the cytokine oncostatin-M. In osteoarthritis, this APA event is altered and so has the potential to contribute to altered cytokine signalling.

P33: MiR-324 affects musculoskeletal biology through the regulation of bone and cartilage maintenance.

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Introduction:
microRNAs are non-coding RNAs that modulate the expression of other RNA molecules, with each microRNA able to target many transcripts. miR-324 is predicted to target thousands of distinct transcripts and has previously been implicated in the regulation of bone and cartilage maintenance - failure of which can lead to common age-related diseases such as osteoporosis and osteoarthritis. We have generated a global miR-324-null mouse model in order to investigate the interaction between Mir324 and musculoskeletal disease in vivo.
Materials and Methods:
Micro-computed tomography (µCT) was used to analyse the bone microarchitecture of limbs from miR-324-null and WT mice of various ages. Histological sections were stained with Goldner's trichome. The function of miR-324 was investigated in bone marrow-derived macrophages, osteoblasts and osteocytes from null and WT mice. Isolated RNA was analysed by qRT-PCR and RNA-seq, and differentially expressed miR-324 target genes validated using 3'UTR-luciferase assays. Alizarin red-S was utilised to stain bone nodules formed by osteoblasts and TRAP-staining was undertaken to stain mature osteoclasts following osteoclastogenesis. Destabilisation of the medial meniscus (DMM) surgery was performed on miR-324-null and WT mice to model osteoarthritis followed by OARSI histopathological scoring. Cartilage damage in aged animals (14 months) was similarly histologically assessed.
Results:
µCT scans of miR-324-null femurs and tibiae revealed that both the cortical and trabecular bone of miR-324-null mice is severely thickened across lifespan. The activities of the two effector bone cells, osteoclasts and osteoblasts, are severely altered. Osteoclastogenesis is severely decreased in miR-324-null bone marrow-derived macrophages, likely as a result of reduced Tnfrsf11a (RANK) expression. Osteoblastic bone formation marker genes are upregulated ex vivo, and mineralisation of the bone nodules produced by miR-324-null osteoblasts is increased. Trichome staining revealed a clear reduction in adiposity in the null mice. Mesenchymal stem cells in AdipOsteo assays were skewed toward osteoblasts when miR-324 was inhibited. Target validation concluded that Runx2 was a direct target of miR-324-5p. Finally, OARSI histopathological scoring revealed that miR-324-null mice displayed increased cartilage damage following either DMM surgery or with age.
Discussion:
By utilising the global miR-324-null mouse model, we have identified that the bones of these animals are thickened, such that miR-324-null mice are osteopetrotic. Furthermore, the lack of miR-324 does not merely affect one class of bone cell, but in fact affects all three primary cell types involved in bone remodelling; miR-324-null mice display decreased osteoclastogenesis, increased osteoblast-mediated bone formation and dysregulation of key osteocyte genes, with differing targets important in each cell-type.
The elucidation of regulatory mechanisms in cartilage and bone, such as those affected in miR-324-null mice, is crucial in the understanding of normal musculoskeletal function, and holds the potential to identify novel pharmaceutical targets for the combating of common musculoskeletal diseases, such as osteoarthritis and osteoporosis.

P34: Time-of-day secretome profiling reveals a novel role for MGP as a timekeeper of myogenic differentiation.

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Introduction:
Skeletal muscle growth and repair are highly regulated processes controlled by interactions between muscle cells and their surrounding niche or microenvironment, including secreted growth factors and extracellular matrix (ECM). The importance of circadian rhythms for skeletal muscle is evident from the altered skeletal muscle phenotypes in mouse models of genetic clock disruption and in human studies. Moreover, muscle-specific genetic clock ablation leads to systemic effects on the entire musculoskeletal system, including bone, tendon and cartilage. Recent work has implicated an important secretory function for skeletal muscle through secreting molecules that affect local muscle ECM in an auto/paracrine manner as well as having endocrine effects on other tissues.
Materials and Methods:
Here we used a quantitative proteomics platform to investigate the secreted factors regulated by time-of-day cues in differentiating murine skeletal muscle cells. These data were further validated using a number of in vitro and in vivo models including real-time bioluminescence imaging, dual luciferase promoter reporter assays, siRNA knowckdowns and growth factor manipulation.
Results:
Our data reveals that 14% of the differentiating muscle cell secretome is regulated in a time-of-day manner, including structural and remodelling ECM components as well as signalling factors. This is accompanied by increased amplitude and rhythmicity of the muscle cell intrinsic clock, in part, reinforced by the feedback from the secreted signalling factors such as pro-anabolic muscle regulators. Moreover, many validated secreted proteins, including a novel skeletal muscle output gene Matrix GLA protein (MGP), are regulated through the transcriptional molecular feedback loops and show circadian expression both in muscle cells in vitro and muscle tissues in vivo. In particular, we show time-of-day Mgp regulation in response to timed growth factor administration or muscle stimulation in vivo. Finally, we reveal dampened amplitude of the molecular clock and altered time-of-day transcriptional regulation of clock-controlled secreted ECM factors in ageing skeletal muscles.
Discussion:
These data implicate an important function for circadian timing in maintenance of appropriate muscle ECM microenvironment during muscle differentiation and necessitate future development of chrono-based interventions for restoring ECM microenvironment in age-related sarcopenia and human muscle-related pathologies.

P35: Understanding the dynamics of the extracellular matrix in the developing human brain.

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Introduction:
It is not completely understood how the human brain develops, but there is recent evidence that extracellular matrix (ECM) plays a key role. The ECM is important for tissue structure and biochemical signalling with many roles in key processes during neurogenesis. However, we do not understand what ECM is present in the developing human brain. RNAseq data showed higher levels of expression of ECM in fetal human brain compared to mouse. Considering that ECM proteins are secreted, this data does not always reflect the actual composition and localization in the brain. We have therefore analysed ECM dynamics by proteomics, using mass spectrometry (MS) across key time points of human brain development and within 2 different cortical areas (progenitors and neurons).
Materials and Methods:
40 samples from 9 post-conception weeks (pcw) to 21 pcw covering critical time points of neurogenesis were collected. Samples were microdissected to separate ventricular and cortical plate regions. 3 different protocols for ECM solubilization and enrichment were compared, using Western blots to validate the extraction of the ECM proteins alongs. Isobaric mass tagging with TMT-pro Plex was use for the MS analysis.
Results:
A final 2-step protocol using the detergent buffer AmBic +ASB-14 followed by further incubation with a buffer GuHCl enabled robust solubilization of ECM proteins. The protocol provided extra extraction of the insoluble proteins. Preliminary analysis of ECM core proteins in the MS trials showed that 19 proteins were extracted with the detergent buffer while another 15 were found with the additional GuHCl buffer step. Most of the proteins (28) were detected when both fractions were combined.
Discussion:
ECM proteins are often thought to be highly insoluble. However, our results show that in human fetal brain a significant fraction of ECM proteins are highly soluble. To extract both the soluble and insoluble ECM fractions, the detergent buffer combined with an extra step using GuHCl buffer yielded the best results. We are now working on the bioinformatic analysis of our MS data to optimise the identification and quantification of ECM proteins.

P36: Distribution and microstructure of elastic fibre system in functionally distinct.

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Introduction:
Tendon consists of collagen fascicles bound together by the interfascicular matrix (IFM). Recent work has shown that the IFM plays an important role in tendon elongation by allowing sliding of fascicles relative to each other. The IFM is more extensive in high-strain energy storing tendons and enriched with elastin. The elastic fibre system consists of varying amounts of elastin deposited on a scaffold of microfibrils (mainly fibrillin -1 and-2). The relative proportions of elastin and fibrillins differ between elastic fibre types, resulting in elastic fibres with different morphologies and mechanical properties. The distribution and the microstructure of elastic fibres in tendon has not been fully examined . We hypothesize that the elastic fibre types differ in energy -storing and positional tendons and become more disordered with ageing.
Materials and Methods:
The energy-storing superficial digital flexor tendon (SDFT) (n=8) and positional common digital extensor tendon (CDET) (n=8) were harvested from young (4–7 years, n=4) and old (18–22 years, n=4) horses. Fascicle and IFM morphology were visualised using H&E stain. Elastic fibre type was investigated using a combination of Millers and Weigert's stains and immuno-staining for elastin, fibrillin-1, and fibrillin-2. Tendon sections were observed using second harmonic generation (SHG) confocal microscopy.
Results:
Thick, mature, elastin-containing elastic fibres were more numerous in the SDFT and predominantly seen in the IFM, where they had an oblique arrangement. The thinnest fibres, likely lacking elastin, were mostly seen in the fascicles and the CDET had significantly more than the SDFT. Fibrillin -2 staining was found in the fascicles and IFM whereas fibrillin -1 was mostly in the IFM. Both fibrillin types were more abundant in the positional CDET. In the IFM, the elastin formed a peri-cellular meshwork and amorphous collagen bridged between elastin fibres. In old tendons, the elastin appeared more disordered and fragmented, especially in the SDFT.
Discussion:
The results suggest that specialised mechanical properties are achieved, in part, by differences in the elastic fibre type and distribution. The thick, elastin-containing fibres in the SDFT IFM allow high strains whereas the positional CDET has more fascicle located, thin, stiffer, fibrillin fibres. Ageing is detrimental to both.

P37: SEMD-JL mutation affects bone structure in the Pro148Leu KIF22 knock-in mice.

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Introduction:
Kinesins are important motor molecules associated with microtubules and involved in cellular cargo, primary cilia, mitosis and meiosis. They are implicated in several ciliopathies, developmental disorders and in mechanotransduction as well as cancer proliferation and metastasis, however, their exact function remains unknown. KIF22 is involved in mitotic spindle formation and chromosome movement. Mutations in KIF22 lead to spondyloepimetaphyseal dysplasia with joint laxity, leptodactylic type (SEMDJL), which presents with severe short stature, joint laxity and multiple dislocations. Despite KIF22 being expressed in cartilage, bone and ligament, its role in these tissues remains unknown. Here we show our initial findings on the impact of KIF22 mutation on bone structure and function.

Materials and Methods:
A knock-in transgenic mouse model of SEMD-JL harbouring a proline (Pro) to leucine (Leu) mutation in the motor domain of KIF22 (KIF22 HET) was generated, in order to decipher the role of KIF22 in skeletal development. The long bones of 3 month old and 16 month old KIF22 HET and wild-type (WT) male mice were analysed by microCT.

Results:
At 3 months there was an increase in trabecular pattern factor (Tb.Pf) of 33% ($p<0.01$) in tibias of KIF22 HET mice compared to WT controls. Cortical analysis showed a more pronounced bone loss with a significant decrease in cortical thickness (12%, $p<0.05$), tissue perimeter (13.5%, $p<0.01$), bone perimeter (13.5%, $p<0.05$) and endosteal perimeter (14%, $p<0.05$) in KIF22 HET mice compared to WT controls. Mean polar moment of inertia (MMI) was decreased by 41% ($p<0.05$) in KIF22 HET mice compared to WT. At 16 months of age KIF22 HET mice had a decrease of 16% in MMI compared to WT mice.

Discussion:
These results show that young adult KIF22 HET mice have a low bone mass phenotype which may be due to an impairment in osteoblast differentiation and/or function. This suggests that KIF22 may play a role in maintaining bone mass.

P38: Acidic pH inhibits the stiffness response in human bone marrow mesenchymal stem cells (BM-MSCs).

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Introduction:
Cells sense mechanical forces from their environment and convert these physical forces into biochemical signals in a process termed mechanotransduction. Importantly, changes in physical force do not occur in isolation, but in concert with chemical factors, a consideration often overlooked. Tissue extracellular pH (pHe) is an important environmental factor known to change during disease and ageing, and often coincident with changes in tissue stiffness. However, the influence of pH on the cellular mechano-response has received little attention. Here, we assessed the proteomic stiffness-response of human BM-MSCs (a cell type often chosen for direct tissue implantation as a therapeutic) cultured on type I collagen coated polyacrylamide gels at extracellular pH (pHe) akin to healthy (pH 7.1), moderately (pH 6.8) and severely (pH 6.5) degenerate musculoskeletal tissues.

Materials and Methods:
Human BM-MSCs isolated from hip joints were expanded on tissue culture plastic and transferred to type I collagen coated polyacrylamide gels of different stiffness (2 and 25 KPa). Following 3 days of culture in medium at pH 7.1, 6.8 or 6.5, cells were trypsinised and processed for label-free mass spectrometry. The peptide abundances were quantified and mapped to proteins using MaxQuant. Differential expression, gene ontology enrichment and reactome pathway analysis were used to assess the systemic response of BM-MSCs to substrate stiffness at different pH.

Results:
At pH 7.1 and 6.8 BM-MSCs were stiffness responsive, increasing proteins in pathways related to regulation of matrix and collagen synthesis. Proteins involved in metabolic pathways, including electron transfer chain and ATP synthesis were down regulated. Differential expression analysis revealed an increase in collagen types I, III and V, and SPARC. Decreases in the metabolic proteins FTL and SOD2 were also observed. At pH 6.5 the mechano-response was inhibited (10 downregulated proteins including TNC and FGL) with no significantly enriched pathways identified.

Discussion:
The mechano-response of BM-MSCs is stiffness and pH-dependent, which has implications for tissue engineering and regenerative medicine (BM-MSCs often suggested for implantation into degenerate tissues with complex physiochemical environments). Further studies are required to elucidate the altered mechanotransduction pathways, which could lead to discovery of novel targets for cell and tissue engineering.

P39: Glaucomatous trabecular meshwork cell-derived extracellular matrix influences differentiation of healthy adult trabecular meshwork stem cells.

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Introduction:
The trabecular meshwork (TM) is an essential tissue in the eye with a complex 3D structure that facilitates aqueous humour outflow and filtration. In POAG patients, TM cellularity decreases along-side an altered and increased accumulation of extracellular matrix (ECM). The resulting disruption to aqueous humour outflow and rise in intraocular pressure leads to retinal ganglion cell dysfunction and irreversible blindness. Adult TM progenitor cells (PET) can contribute to the proliferation response seen with externally induced damage but do not do so with increasing age and POAG. We investigate the influence of glaucomatous TM derived ECM on the differential ability of PET.
Materials and Methods:
Primary TM cells (pTM) were cultured from dissected human donor TM tissue (one donor, aged 28 years old). Primary cultures were characterised by immunofluorescent staining for myocilin expression after 7 days treatment with 100nM dexamethasone, and other TM markers, CHI3L1 and TIMP3. PET were isolated over 7 days by sphere culture, a method encouraging growth of stem cells whilst initiating apoptosis of differentiated cells. pTM cells, glaucomatous (GTM3) and normal (NTM5) immortalised TM cell lines were cultured for 5-7 days to allow them to deposit ECM, before the ECM was decellularized by lysis with 2% ammonium hydroxide solution. Spheres were differentiated on ECM deposited by pTM cells, GTM3 and NTM5 for 7 days.
Results:
TMSC spheres successfully attached to tissue culture plastic (TCP) and GTM3, NTM5 and pTM ECM coated wells within 24 hours. Irrespective of ECM coating, spheres differentiated into cells with TM cell morphology (fibroblast like) and expressed TM markers MYOC, MGP, TIMP3 and CHI3L1 as determined by q-PCR.
Discussion:
Initial outgrowth of differentiated cells from spheres appears greater for those differentiated on GTM3 ECM compared to TCP, NTM5 ECM and pTM ECM.

P40: Overexpression of asporin promotes chondrogenesis and stimulates mineralisation of ATDC5 cells.

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Introduction:
Asporin (ASPN) is a small leucine-rich proteoglycan (SLRP) with a unique N-terminus of 13 aspartic acid residues and ability to bind calcium. Asporin has been shown to be upregulated in osteoarthritic cartilage, and localised to the developing bone. ATDC5 cells are a commonly used cell line to model chondrogenesis, more recently they have also been shown to be capable of modelling cartilage mineralisation.

Materials and Methods:
Asporin was overexpressed in ATDC5 by ASPN ORF expression vector (GenScript). ATDC5 cells overexpressing asporin (OE) were differentiated with chondrogenic medium without betaglycerophosphate (βGP) for chondrogenesis and with βGP to drive ATDC5 mineralisation for 21 days. Cell monolayer was stained with Alcian blue for proteoglycan deposition (without βGP) and 40mM Alizarin Red for calcium deposition (with βGP). Gene expression was assessed by RT-qPCR.

Results:
In the chondrogenic model Col10a1, a marker of terminal chondrocyte differentiation, was upregulated in the OE ATDC5 at day 21 compared to the WT control. Col2a1, Acan and Sox9 (markers of chondrogenesis) were also upregulated significantly during chondrogenesis. Alcian blue staining was increased in the ASPN OE ATDC5 monolayer by day 14 compared to day 1. Mmp13 was significantly upregulated in the mineralising ASPN OE. Addition of βGP successfully induced mineralisation, furthermore calcium deposition was accelerated with significantly more Alizarin Red staining was present by day 7 OE cells, up to day 35 where the difference was indistinguishable.

Discussion:
Overexpression of asporin accelerated chondrogenesis and mineralisation. Previous work has shown Asporin to be an inhibitor of chondrogenesis however more recently asporin has been demonstrated to activate the TGFβ1 signalling pathway. In this study overexpression of Asporin upregulated Col2a1, Acan, Sox9 and Col10a1 during ATDC5 chondrogenesis. Furthermore, overexpression drove production of a glycosaminoglycan (GAG)-rich ECM. Asporin overexpression accelerated ATDC5 mineralisation and Mmp13 expression. Interestingly, introduction of MMP13 has been shown to stimulate the calcification of the cartilage matrix in previous studies.
The role of asporin in the developing long bones is not fully understood, however the upregulation of Mmp13 and increased Alizarin Red staining in the asporin overexpressing mineralising ATDC5 cells indicates a role in cartilage calcification and scaffold degradation in endochondral ossification.

P41: A novel treatment for scleroderma fibrosis: The CCN3-based peptide BLR-200 impairs bleomycin-induced skin fibrosis.

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Introduction:
Of the CCN family of matricellular proteins, CCN2 promotes, whereas CCN3 inhibits, in vivo models of fibrosis. A proprietary, synthetic peptide (BLR-200) produced by BLR Bio and based on an amino acid sequence identified within CCN3 has been shown to specifically mimic CCN3's antifibrotic activity. However, the mechanism underlying BLR-200's antifibrotic activity is only now beginning to be elucidated. Previously, we have shown that collagen-lineage fibroblasts are essential for inflammation-induced skin fibrosis. However, the specific subsets of collagen-lineage fibroblasts activated in response to inflammation-induced skin fibrosis are unidentified. Moreover, whether BLR-200 blocks their activation is unknown.
Materials and Methods:
Three-week-old Col1A2-Cre(ER)T/0; mT/mG C57Bl/6j mice were subcutaneously injected with tamoxifen for 5 consecutive days to label collagen-lineage fibroblasts with green fluorescent protein (GFP) and subsequently injected with PBS or bleomycin daily for 10, 21 or 28 days. Mice were injected every other day with BLR-200 or scrambled sequence control peptide. GFP-positive cells were isolated by fluorescence-activated cell sorting (FACS) and subjected to scRNAseq. Six-week old C57Bl/6j mice were similarly treated. Intact skin was isolated, and subjected to hisological, proteomic, and bulk RNAseq/real-time polymerase chain reaction (RT-PCR) analyses.
Results:
In response to bleomycin, inflammatory subsets of collagen-lineage fibroblasts were activated, according to scRNAseq analysis. BLR-200 impaired this response, by selectively blocking induction of IL-6 and egr1, but not of IL-1 or AP-1 family members. Histological analysis revealed that BLR-200 broadly impaired the ability of bleomycin to induce skin thickness, collagen production and myofibroblast differentiation ($N=8$, $p<0.05$). Proteomic and RNA analyses confirmed that BLR-200 impaired pro-fibrotic cohorts of proteins and RNAs induced by bleomycin.
Discussion:
BLR-200 impairs the ability of fibroblasts to respond to inflammatory insults and thus may represent a novel, anti-fibrotic therapeutic approach.
THIS ABSTRACT CONTAINS CONFIDENTIAL INFORMATION

P42: Molecular analyses identify WWP2 as the target gene of an osteoarthritis risk SNP whose functional effects have origins during cartilage development.

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Introduction:
Over 100 osteoarthritis-risk single nucleotide polymorphisms (SNPs) have so far been detected, primarily via genome-wide association studies (GWAS). Most of the risk-conferring alleles are predicted to modulate the expression of a target gene. Osteoarthritis GWAS signals often correlate with cartilage DNA methylation (DNAm) at CpG dinucleotides (CpGs), forming methylation quantitative trait loci (mQTLs). Changes in DNAm can act as a functional intermediary between SNP and target gene. A growing body of evidence suggests that developmental factors contribute to osteoarthritis susceptibility. Recent GWASs identified the osteoarthritis SNP rs34195470 (A>G), which resides within WWP2. This gene encodes an E3 ubiquitin ligase abundantly expressed in cartilage and known to play a role in chondrogenesis. WWP2 is also the host gene of miRNA-140, a known regulator of chondrocyte activity. We previously reported mQTLs at two WWP2 CpGs 3.9kb downstream of rs34195470. Here, we elucidate the functional target of osteoarthritis-risk marked by rs34195470 and compare WWP2 expression and DNAm profiles between foetal and osteoarthritis cartilage.
Materials and Methods:
Cartilage DNA was genotyped at rs34195470 and DNAm quantified at 16 CpGs using pyrosequencing. Guide RNAs (gRNAs) targeting these CpGs were cloned into plasmids encoding dCas9-DNMT3a and nucleofected into TC28a2 chondrocytes. Nucleic acids were extracted after 72 hours. RNA from cartilage samples and TC28a2 chondrocytes was reverse transcribed and expression of WWP2 and miRNA-140 measured by RT-qPCR.
Results:
mQTLs were identified at 5/16 CpGs in foetal cartilage (P=0.0096-0.0266), and 14/16 CpGs in osteoarthritis cartilage (P=0.0010-0.0476), where the osteoarthritis risk-conferring allele G of rs34195470 correlates with increased DNAm, marking a differentially methylated region (DMR). Osteoarthritis cartilage is hypermethylated with increased expression of WWP2 compared to foetal (P=0.0070). Increased DNAm at the DMR following dCas9-DNMT3a modulation correlated with significant upregulation of WWP2 (P=0.0050-0.0340). miR-140 expression did not change significantly (P>0.05).
Discussion:
We have identified an osteoarthritis-associated DMR, in which increased DNAm correlates with disease risk, that is active during development. This region becomes hypermethylated with age, coinciding with increased expression of WWP2. Epigenetic modulation of the DMR increased expression of WWP2 in TC28a2 chondrocytes, suggesting that it is the functional target of the rs34195470 association signal.

P43: Ben Issa

Introduction:
Tumour metastasis accounts for more than 90% of all cancer related mortalities and remains a major focus of cancer research. The process by which tumour cells escape from their primary site to form a secondary tumour in a distant organ is known as epithelial-mesenchymal transition (EMT). EMT is regulated by tumour microenvironment, mainly through matrix stiffness and availability of chemokines/cytokines, which accounts for the poor prognosis and chemotherapy resistance in cancer patients. We hypothesise that matrix stiffness defines the malignancy and biological features of metastatic and non-metastatic carcinoma cells.
Materials and Methods:
Two isogeneic breast adenocarcinoma cell lines; MDA231 and MDA231-shZEB1 (ZEB1 knock down) were seeded on soft (1kPa) and stiff (40kPa) polyacrylamide hydrogels to investigate the effect of matrix stiffness on cellular properties. This was done using DNA proliferation assay, directional and non-directional migration assay, cell area calculation macro by FijiImageJ, and EMT marker analysis using PCR. We also investigated the displacement exerted by these cells on soft polyacrylamide hydrogels containing fluorescent beads as a readout of force applied towards substrate. This was done using a Matlab code that spatially cross-correlates sequential time-lapse images evaluating gel deformation caused by these cells.
Results:
MDA231 cell area increased significantly as a function of matrix stiffness ($p<0.0001$). In addition, there were qualitative difference in morphology, with cells on soft materials appearing rounded and confined and those on stiff materials with a spread elongated morphology and defined stress fibres. Increased stiffness was also associated with increased cell proliferation and migration ability, $p<0.0001$. Furthermore, we found that epithelial cells exert larger displacement than mesenchymal cells and cells seeded at high density (5000 cell/cm3 or higher) exert lower displacement compared ($p<0.001$).
Discussion:
Our observations that matrix stiffness upregulates metastatic properties of breast cancer cells indicate that stiffness of tumours is a regulating factor of tumour metastasis. This agrees with previous studies reporting that ECM stiffness promotes cell growth, survival, and migration. Additionally, displacement data suggests that neighbouring cells contribute to the feel of stiffness, probing them to react in the same way as on stiff substrates. It also highlights the role of seeding density in the mechanosensitivity of the cells affecting how hard/stiff the underlying substrate feels and, therefore, how cells respond.