BSDB

British Society for Matrix Biology

1st Joint Meeting of the British Societies for Matrix Biology and Developmental Biology

The Musculoskeletal System
from Development to Disease

1.-3. September 2014
University of East Anglia, Norwich

Confirmed Speakers:

Mike Briggs (Newcastle)
Madeleine Durbeej (Lund)
Chrissy Hammond (Bristol)
Christine Hartmann (Münster)
Gabrielle Kardon (Salt Lake City)
Cay Kielty (Manchester)
Veronique Lefebvre (Cleveland)
Malcolm Logan (London)
Paul O'Higgins (Hull)
Andy Pitsillides (London)
Ronen Schweitzer (Portland)
Simon Tew (Liverpool)
Linda Troeberg (Oxford)
Eli Zelzer (Weizmann)

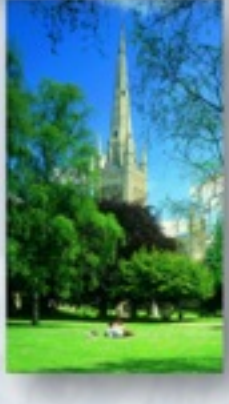
Additional speakers will be selected from abstracts

Keynote Speakers:

Tom Rando (Stanford)
David Glass (Novartis)

Major Topics:

Signaling
Development
Stem Cells
Mechanobiology
Anatomy
Human Genetics
Pathology



Organizers:
I. Clark, A. Münsterberg
G. Riley, U. Mayer
T. Vincent
Further information: www.bsmb.ac.uk/bsmb-bsdb

THE MUSCULOSKELETAL SYSTEM, FROM DEVELOPMENT TO DISEASE

1st joint meeting of the British Society for Matrix Biology (BSMB) and the British Society for Developmental Biology (BSDB)

The University of East Anglia, Norwich Research Park, Norwich UK
1st-3rd September 2014

Monday 1st September

11:00 Registration opens

13:00 **Opening remarks by the organizing committee**

13:10-13:50 **The MOD-ISDB Keynote Lecture**
Tom Rando (Stanford)
"Molecular Regulation of Stem Cell Quiescence"

Session 1: **Signaling and Development**
Chair: Andrea Münsterberg

13:50-14:05 Anne-Gaelle Borycki (Sheffield) - **Poster 1**
"Remodeling at the satellite cell basal lamina during muscle regeneration"

14:05-14:35 Gabrielle Kardon (Utah)
"Whole or hole? Development of the diaphragm and congenital diaphragmatic hernias"

14:35-14:50 Dylan Sweetman (Nottingham) - **Poster 2**
"FGF18 and retinoic acid regulate timing of limb myoblast differentiation"

14:50-15:30 **Tea/Coffee, Posters & Exhibits**

15:30-16:00 Malcolm Logan (NIMR, London)
"The role of muscle connective tissue in morphogenesis of the limb soft tissues"

16:00-16:15 Susanne Dietrich (Portsmouth) **– Poster 3**
"The role of Engrailed for epaxial-hypaxial muscle development and innervation, and for the evolution of vertebrate three-dimensional mobility"

16:15-16:30 Gi Fay Mok (Norwich) - **Poster 4** Prize Entrant
"The coordinated regulation of BAF60 variants by miR-1/206 and miR-133 clusters stabilises myogenic differentiation during embryogenesis"

16:30-17:00 Christine Hartmann (Münster)
"Wnt-signaling and trabecular bone formation"

17:00-17:15 Clare Thompson (QMU London) – **Poster 5** Prize Entrant
"Lithium chloride triggers primary cilia elongation and inhibits hedgehog signalling in articular chondrocytes"

17:15- 17:30 Sue Kimber (Manchester) – **Poster 6**
"Differentiation of Chondrocytes from Pluripotent Stem Cells for Cartilage repair"

17:30-20:00 **Poster Session and Evening Reception**

Tuesday 2nd September

Session 2: **Mechanobiology and Anatomy**
Chair: Graham Riley

8:30-9:00 Eli Zelzer (Weizmann Institute) –
"A novel mechanism of spontaneous fractured bone regeneration"

9:00-9:15 Cornelia Stein (Cologne) – **Poster 7**
"Analysis of the role of Hemicentin proteins during mouse and zebrafish development"

9:15-9:30 Angela Yiu (King's College, London) – **Poster 8** Prize Entrant
"Fat-Hippo Signalling in Bone Development"

9:30-10:00 Paul O'Higgins (York)
"*Virtual anatomies: how computers are transforming studies of musculoskeletal form and function."*

10:00-10:15 Rebecca Rolfe (Dublin) – **Poster 9**
"The impact of mechanical stimulation from muscle contractions on ossification and joint formation during mouse development: exploring developmental mechanisms disturbed in the absence of contractions."

10:15-10:30 Mario Giorgi (London) – **Poster 10** Prize entrant
"The role of fetal movement in prenatal hip joint morphogenesis"

10:30-11:00 Chrissy Hammond (Bristol)
"Unpicking the relationship between mechanical loading and genes in shaping the developing joint."

11:00-11:45 **Tea/Coffee, Posters & Exhibits**

11:45-12:15 Ronen Schweitzer (Portland)
"Regulation of tendon growth and maturation"

12:15-12:30 Herbert Tempfer (Salzburg) – **Poster 11**
"Is there a population of neural crest derived murine tendon cells?"

12:30-13:00 Andy Pitsillides (Royal Veterinary College)
"Limb growth and joint formation: move-it, move-it"

13:10-14:00 **Lunch**

Session 3: **Human Genetics and Pathology**
Chair: Ulrike Mayer

14:00-14:30 Mike Briggs (Newcastle)
"The role of ARMET and CRELD2, two recently described ER-stress induced proteins, in chondrocyte function and cartilage pathobiology"

14:30-14:45 Qing-Jun Meng (Manchester) – **Poster 12**
"The tissue specific functions of the circadian clocks: implications in diseases and tissue regeneration"

14:45-15:00 Pradeep Kumar Sacitharan (Oxford) – **Poster 13** Prize Entrant
"Deletion of SirT1 drives joint inflammation and suppresses anabolic chondrocyte gene expression"

15:00-15:30 Madeleine Durbeej (Lund)
"Laminin-deficient muscular dystrophy: pathogenesis and development of treatment"

15:30-16:30 **Tea/Coffee, Posters & Exhibits**

16:30-17:00 Linda Troeberg (Kennedy Institute)
"*Regulation of cartilage extracellular matrix turnover by the endocytic receptor LRP-1"*

17:00-17:15 Carole Proctor (Newcastle) – **Poster 14**
"Investigating molecular mechanisms of ageing cartilage using computer simulation models"

17:15-17:45 **Lecture sponsored by IJEP**
Veronique Lefebvre (Cleveland)
"Rivalry between Sox proteins define your skeleton"

18:30 **Departure by Coach to St. Andrews' Hall for Conference Dinner**

Wednesday 3rd September

Session 4: **Transcriptional and Epigenetic Regulation**
Chair: Ian Clark

8:30-9:00 Cay Kielty (Manchester)
"*Fibrillin microfibrils: genotype-to-phenotype in the fibrillinopathies"*

9:00-9:15 Matt Barter (Newcastle) – **Poster 15**
"Long non-coding RNAs in chondrocyte development and cartilage"

9:15-9:30 Linh Lee (Norwich): - **Poster 16** Prize entrant
"The miR-29 family in osteoarthritis"

9:30-10:00 Simon Tew (Liverpool)
"Post transcriptional gene regulation in human chondrocytes"

10:00-10:45 **Tea/Coffee, Posters & Exhibits**

10:45-11:15 **BSMB John Scott Lecture (Young Investigator Award)**
Blandine Poulet (UC London)
"Mouse models of osteoarthritis progression"

11:15-12:00 **Developmental Dynamics Keynote** Lecture
David Glass (Novartis)
"Signaling pathways that regulate skeletal muscle size and function"

12:00 **Closing Remarks by Tonia Vincent (on behalf of the organizing committee)**

12:15 **Lunch Bag and Departure**

INTERNATIONAL JOURNAL OF Experimental Pathology

Int. J. Exp. Path. (2015), 96, A1–A30

Joint meeting of British Societies for Matrix Biology and Developmental Biology '*The musculoskeletal system; from development to disease*'

Autumn 2014 Meeting Report

Organised jointly by Andrea Munsterberg from the British Society for Developmental Biology (BSDB) together with Uli Mayer, Ian Clark, Graham Riley and Tonia Vincent from the British Society for Matrix Biology (BSMB).

Report written by Pradeep Sacitharan (Oxford).

This inaugural joint meeting took place at the University of East Anglia (Norwich) from 1st to 3rd September 2014. Some 130 delegates took part. This report will highlight some of the key findings which were conveyed in the four main sessions: signalling and development, mechanobiology and anatomy, human genetics and pathology and transcriptional and epigenetic regulation.

Signalling and development

Thomas Rando (Stanford) outlined a novel form of long lasting cellular memory in stem cells. This has been coined the "GAlert state" in which a past stimulus results in the cell response being heightened. This state allows stem cells to respond and adapt more rapidly and efficiently to newer challenges. He also demonstrated the existence of signals which communicate from an injured tissue to stem cell compartments across an organism.

Gabrielle Kardon (Utah) described her group's work in determining that pleuroperitoneal folds are the original source of connective tissue in the diaphragm and how mutations of Gata4 in these folds caused congenital diaphragmatic hernias.

Dylan Sweetman (Nottingham) showed that retinoic acid inhibited early myoblast differentiation. However, retinoic acid signalling reduces as the limb grows and FGF18 then induces myoblast differentiation.

Claire Thompson (London) demonstrated lithium chloride stimulation of primary cilia elongation and inhibition of hedgehog signalling in articular chondrocytes. Finally, Sue Kimber (Manchester) explained a new protocol to induce differentiation of pluripotent stem cells towards chondrocytes, which were shown to repair osteochondral defects in rats.

Mechanobiology and anatomy

Cornelia Stein (Cologne) showed hemicentin proteins are required at the sites of mechanical forces. Using novel zebrafish and murine models she outlined the presence of hemicentin proteins in myotendinous junctions and increases in their expression during wound healing. Cheuk Ting Yiu (London) explained the importance of the Fat-Hippo signalling cascade in bone development. She showed increased Yap activity in Fat4-/- and Dchs1-/- calvaria and limb osteoblasts and suggested that Yap activity is downstream to Fat4-/- and contributes to osteoblast differentiation leading to the skeletal phenotypic defect in these mice. Paula Murphy (Dublin) outlined 84 key genes linked with the cytoskeleton and involved in converting a mechanical stimulus into a transcriptional response. Among these, the WNT signalling pathway was particularly important. Herbert Tempfer (Salzburg) using transgenic mice then showed elegantly that murine tendon cells express neuron-associated markers consistent with a potential neural crest origin.

Human genetics and pathology

Quing-Jung Meng (Manchester) gave an insightful talk on his group's work which has identified hundreds of circadian rhythmic genes in cartilage and tendon tissue. Global or conditional knockout models of these clock genes resulted in pathologies such as cartilage degeneration and calcification of tendons. Madeleine Durbeej (Lund) presented data which shed light on the elusive pathogenesis of congenital muscular dystrophy type 1A. Using dy3k/dy3k mouse models she demonstrated the role of the autophagy and ubiquitin-protease systems in promoting the disease. Moreover, mice displayed a less dystophic phenotype when these cellular systems were inhibited.

Carole Proctor (Newcastle), using computer simulation models, showed the overall contribution of negative feedback loops in controlling increases in the number of TGFβ type 1 and ALK1/5 receptors during aging in cartilage, which increased MMP-13 expression and collagen degeneration. Veronique Lefebvre (Cleveland) closed the day by showing that the SoxC trio (Sox4/11/12) stabilised beta-catenin and repressed Sox9 expression, which secures the non-chondrocytic fate of mesenchymal progenitor stem cells; this is the opposite effect to that of the chondrogenic Sox5/6/9 trio. Thus, these sets of trios decide the fate of mesenchymal progenitor stem cells and aid in developing a full mature skeleton.

Pathology & transcriptional & epigenetic regulation

The last day of the meeting started with Cay Kielty (Manchester) presenting new insights into fibrillinopathies and

microfibril biology. ADAMTS-10 and not ADAMTS-6 was shown to regulate microfibril assembly by molecular interactions with heparin/heparan sulphate and other molecules including LTBP-1.

Simon Tew (Liverpool) gave an informative talk on post-transcriptional regulation in chondrocytes, highlighting 395 mRNAs with different decay rates in OA and non-OA chondrocytes; most differentially regulated genes were nuclear, involved in apoptosis and embryonic development.

This year's BSMB John Scott lecture was given by Blandine Poulet (London). She outlined her work on deducing the mechanisms which control osteoarthritis progression using the mechanical post traumatic model she had developed and a spontaneous model of OA. Overall the data suggested OA progression is dependent on the initiating factor–hence the importance of using different models to study the process.

The meeting was concluded by David Glass (Novartis) describing signalling pathways involved in skeletal muscle atrophy. Muscle RING finger 1 and Atrogin were shown to be transcriptionally upregulated in atrophy. Data was also presented using a novel human anti-activin receptor II antibody, bimagrumab, which increased muscle mass in mice and protected muscles from glucocorticoid-induced atrophy and weakness.

Acknowledgements

PS gratefully acknowledges receiving a reporter award from the BSMB which made it possible to attend, present work and participate in the meeting.

Meeting Abstracts

FGF18 and retinoic acid regulate timing of limb myoblast differentiation

D. Sweetman[1], G. F. Mok[2], R. Cardenas[1] and H. Anderton[1]
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Introduction Limb muscles are derived from Pax3 expressing myoblasts that delaminate from the hypaxial somite and migrate into the developing limb bud. They populate two distinct regions of the limb bud, the dorsal and ventral muscle masses, and only then do they begin to differentiate and express muscle specific markers such as Myf5 and MyoD. However the signals regulating this process have not yet been identified.
Materials and Methods Chicken embryos are grafted at precise developmental stages with beads soaked in recombinant growth factors, retinoic acid or small molecule inhibitors of specific signaling pathways. Myogenic gene expression is then measured by whole-mount in situ hybridisation.
Results FGF18 is expressed in limb bud mesenchyme adjacent to the differentiating myoblasts and can interact with FGFR4, which is known to affect limb bud myogenesis. We show that FGF18 beads grafted into chicken embryo limb buds *in ovo* induce early expression of Myf5 and MyoD along with downregulation of Pax3 and that this is mediated by ERK MAP kinase activation. We also show that this activity of FGF18 can be blocked by treatment with retinoic acid. Interestingly at early stages of limb development (HH19) Myf5 is induced by FGF18 but MyoD is not while at later stages (HH21) both are induced. However in the presence of a retinoic acid antagonist it is possible to induce MyoD in HH19 limbs. This suggests an interaction between these signaling pathways which regulates the onset of limb myoblast differentiation.
Discussion We propose a model where FGF18 in the limb bud mesenchyme and retinoic acid from the flank interact to control the timing of limb myoblast differentiation. In early limb buds RA inhibits myoblast differentiation but as the limb grows and RA signaling is reduced FGF18 is able to induce expression of myogenic determination genes and initiate differentiation.

The impact of mechanical stimulation from muscle contractions on ossification and joint formation during mouse development: exploring developmental mechanisms disturbed in the absence of contractions.

P. Murphy[1], R. Rolfe[1], C. Shea[1] and A. Saha[1]
[1]*School of Natural Sciences, Trinity College Dublin, Dublin 2, Dublin 00002, Irish Republic*

Introduction Mechanical stimulation due to muscle contractions is necessary for correct bone and joint formation in utero. We previously took two approaches to studying the influence of muscle contractions on skeletal development *in vivo*. In the chick system we used neuromuscular blocking to reduce muscle contractions in ovo. In the mouse system, mutant lines in which no skeletal muscle forms (Myf5nlacZ/nlacZ: Myod-/- and Pax3sp/sp) were used to assess skeletogenesis in the absence or reduction of muscle contractions. We revealed abnormal long bone ossification, joint formation and alterations in the expression patterns of developmental regulatory genes in an altered mechanical environment in both systems. The specific changes observed correlated with the mechanical stimuli in operation, predicted by Finite Element Analysis.
Materials and Methods Two major challenges are the focus of current work: 1) uncovering the mechanistic basis of how

biophysical stimuli are integrated with molecular regulation of cellular differentiation and tissue morphogenesis; 2) Applying the knowledge gained from the developing embryo to improving *in vitro* regimes to stimulate progenitor cell differentiation for transplantation therapies. To address challenge 1 we used differential screening methods (microarray and whole transcriptome sequencing) to identify differentially expressed genes between muscle-less (Spd) and control embryonic humerus tissue (Theiler stage 23). Gene expression analysis and functional testing are being used to screen genes.

Results We found 680 genes down-regulated and 452 genes up-regulated in muscle-less embryos compared to littermate controls (at least two-fold; corrected P-value <0.05). Analysis of Gene Ontology annotations revealed strong enrichment of genes associated with particular biological processes including development and differentiation, cytoskeletal architecture and cell signalling. Among cell signalling pathways, the most strongly disturbed was Wnt signalling. The identification of 84 genes associated with the cytoskeleton indicates candidate genes that are both mechanoresponsive and potentially involved in mechanotransduction, converting a mechanical stimulus into a transcriptional response.

Discussion This work identifies key developmental regulatory genes impacted by altered mechanical stimulation and sheds light on the molecular mechanisms that interpret mechanical stimuli during skeletal development. In particular it highlights the Wnt signalling pathway as a potential point of integration of mechanical and molecular signalling and cytoskeletal components as mediators of the response.

Raman spectroscopy reveals evidence for early bone changes in osteoarthritis

J. G. Kerns[1], K. Buckley[2], P. D. Gikas[4], H. L. Birch[4], I. D. McCarthy[4], R. Keen[3], A. W. Parker[2], P. Matousek[2] and A. E. Goodship[4]

[1]*Lancaster Medical School, Furness College, Lancaster University, Lancaster LA1 4YG, UK,* [2]*Laser Facility Research Complex at Harwell STFC Rutherford Appleton Laboratory Harwell, Oxford OX11 0FA, UK,* [3]*Royal National Orthopaedic Hospital, Brockley Hill, Stanmore HA7 4LP, UK,* [4]*Institute of Orthopaedics and Musculoskeletal Science, University College London, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore HA7 4LP, UK*

Introduction Osteoarthritis (OA) is a common, debilitating disease of joints involving degeneration of cartilage and bone. It has been suggested that subtle changes in the molecular structure of subchondral bone may precede morphological changes in the osteoarthritic joint. There are two arguments for the mechanical initiation of osteoarthritis: (1) Increased subchondral bone stiffness due to increased load on the joint; (2) Changes in bone biochemistry towards homotrimeric collagen. The samples were analysed with Raman spectroscopy, peripheral quantitative computed tomography (pQCT) and chemical analysis, specifically comparing both medial and lateral compartments. The aim of our study is to explore the hypothesis that there are changes in the bone matrix chemistry of OA subchondral bone compared to matched individuals unaffected by OA.

Materials and Methods Samples were acquired (with ethical approval) from human tibial plateaus with established medial compartment osteoarthritis (n = 10), non-OA from amputees (n = 5) and non-OA aged-matched cadaveric tissue (n = 5). pQCT was used to calculate volumetric bone mineral density, and chemical analysis provided collagen alpha chain ratios for each sample. Raman spectra were acquired from the subchondral bone with an 830 nm laser (Renishaw, UK). Mineralisation ratios were calculated as well as multivariate analysis to assess variance across the spectral range (750–1800 cm^{-1}).

Results The subchondral bone of the medial side of the OA samples had a greater vBMD ($P = 0.05$) and is thicker than the lateral side. The non-OA specimens had the same thickness on both sides of the tibial plateau with some differences in density. The initial Raman spectral results show there is no spectral difference between the two sides of the plateau in OA or in the non-OA samples (Figure 1). However, there were differences in Raman spectra between the OA and non-OA samples ($P = 0.02$), regardless of compartment and exhibited differences in collagen chemistry.

Discussion These results support the theory that there is a biochemical difference between OA and non-OA subchondral bone. Future efforts will assess Raman spectroscopy for characterising and detecting osteoarthritis during its early subclinical phase. The results demonstrate that Raman spectroscopy should be further developed as a future tool to provide screening for early detection of joint degeneration based on correlating molecular-specific modifications in the subchondral bone.

Differentiation of chondrocytes from pluripotent stem cells for cartilage repair

S. J. Kimber[1], A. Cheng[1], Z. Kapacee[1], T. E. Hardingham[1], N. Bates[1] and T. Wang[1]

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Introduction Both human embryonic (hESC) and induced pluripotent (iPSC) stem cells have tremendous promise for regenerative medicine because they self-renew indefinitely and form cell types of all three germ layers. However their plasticity can be a problem and one barrier to their use is the difficulty in targeting differentiation only to one desired cell type. We devised a protocol to target differentiation of hESCs towards chondrocytes with high efficiency in 5/6 lines tested. This 3-stage protocol induces both hESCs and iPSCs to differentiate through developmental intermediate stages using ECM substrates and chemically-defined medium to give 94–97% SOX 9 (key chondrogenic transcription factor) positive cells, expressing COL2A1 (the characteristic collagen of hyaline cartilage) in Safranin O positive aggregates.

Here we analyse the differentiation pathway and refine the protocol.
Materials and Methods Growth factor (e.g. alternative BMPs) supplementation was used to enhance chondrocyte differentiation. Cells underwent ChIP-qPCR analysis to determine SOX9 transcriptional regulation. Infection with a proteasome ubiquitin-YFP reporter evaluated ER stress. Illumina RNA seq was conducted for pathway analysis. Osteochondral defects in the hind limb joint of immunocompromised RNU rats were implanted with hESC-chondrocytes in fibrin gels, and monitored histologically.
Results Although extensive gene characterisation reveals reproducible progression of hESC through mesendoderm, and mesoderm-like stages, to chondrogenic aggregates, the cells were immature and did not produce equivalent amounts or organisation of matrix as found in hyaline cartilage. Through interrogation of RNAseq data (two hESC-lines) we identified matrix synthesis and assembly pathways as targets for modulation by inhibition or knock-down. By subtle modulation of the growth factor milieu we have now improved chondrocyte maturity. During hESC-chondrogenesis SOX9 was transcriptionally-paused until day 5 at which time transcription is released and although ER stress increased as the cells differentiated, proteasome function was maintained suggesting this is not detrimental to ECM protein synthesis. We show that hESC-derived chondrocytes can repair osteochondral defects in rats, giving good quality hyaline-like cartilage matrix in which human cells were detected at 12 weeks.
Discussion Our protocol is efficient, scalable and reproducible, generating further understanding of the mechanisms involved in hESC differentiation to chondrocytes and opening up the potential for translation to disease models and clinical therapies.

Investigating hedgehog signalling via the primary cilium in skeletal muscle development and regeneration

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Introduction The primary cilium has recently been recognised as an essential regulator of the Sonic hedgehog (Shh) signalling pathway. Mutations that disrupt cilia function in humans can cause conditions known as ciliopathies. In chick and mouse models of ciliopathies there are a wide range of phenotypes observed, including polydactyly, craniofacial defects and polycystic kidneys. The Shh pathway is vital for a wide variety of developmental processes, including embryonic muscle development, with recent evidence suggesting it may also play a role in adult muscle regeneration. Shh signalling occurs via the primary cilium. Our studies focus on the Talpid3 gene, which encodes a centrosomal protein required for primary cilia formation. The Talpid3 loss-of-function mutant has perturbed ciliogenesis and displays many of the phenotypes that are typically associated with ciliopathies. Talpid3 mutants have defects in Shh signalling and the processing of Gli transcription factors is affected in structures such as the developing limb buds and the neural tube. However, the role of Talpid3 in muscle development and regeneration remains unknown.
Materials and Methods To study the role of Talpid3 in embryonic myogenesis we generated conditional knock out mice using Pax3-Cre mice. The role of Talpid3 in adult muscle regeneration will be investigated using a cardiotoxin injury model, either by co-injection of an adenovirus expressing Cre recombinase, or by crossing Talpid3 floxed mice to a mouse carrying an inducible Pax7-CreERT2 allele. In situ hybridisation and histochemistry on cryosections is used to study effects of Talpid3 loss on embryonic and adult myogenesis.
Results Via in situ hybridisation, we observe disrupted Myf5 expression in myoblasts in somites and limbs in Pax3-Cre Ta3*f/f* mice. Regeneration was affected in adeno-Cre injected Ta3*f/f* tibialis anterior (TA) muscle fibres after cardiotoxin injury. Experiments using Pax7-Cre Ta3 mice are in progress.
Discussion Our preliminary data suggest that Talpid3 is important for embryonic myogenesis and has a role in adult muscle regeneration.

Fibrillin–1 is expressed in cartilage and mutation leads to OA development

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[1]*University College London Centre for Rheumatology and Connective Tissue Diseases, Division of Medicine Royal Free Campus, Rowland hill street2nd floor, London NW3 2PF, UK,* [2]*Royal Veterinary College Comparative Biomedical Sciences Royal College Street, London NW1 0TU, UK,* [3]*University College London, Department of Cell and developmental biology, Gower street, London WC1E 6BT, UK*

Introduction Fibrillin-1 is an extracellular matrix protein found in elastic fibres. One of its main roles is to control growth factor bioavailability, which play vital functions in joint homeostasis. Fibrillin-1 mutations, as in Tight Skin mice (TSK), can increase TGFβ signalling, and leads to skin fibrosis, myocardial hypertrophy, marfan-like skeletal phenotype and lung emphysema. The aim of this study is to describe the expression pattern of Fibrillin-1 in mouse knee joints, and to characterise the effect of Fibrillin-1 mutations in ageing-induced OA development in TSK mice.
Materials and Methods Immunohistochemistry for Fibrillin-1 was performed in CBA and Str/ort mice (spontaneous OA), and in trauma-induced OA. Knees of 60–80 week-old TSK and littermate male mice were microCT scanned, ectopic calcification and subchdondral bone thickness analysed. Six μm serial coronal sections were scored for OA severity.
Results Fibrillin-1 was found in the pericellular matrix of chondrocytes in both the growth plate (resting and hypertrophic cells) and in the uncalcified articular cartilage in

healthy CBA mice. During the development of OA in Str/ort mice, Fibrillin-1 immunolabelling was decreased, in particular around lesions; in contrast, mRNA expression from the articular cartilage of Str/ort mice was increased with OA development. Fibrillin-1 protein levels were also significantly decreased in response to mechanical trauma *in vivo*.

TSK mice microCT images showed important ectopic calcification in various ligaments, as well as osteophyte formation on the margins of the joints. Preliminary data suggest that articulate cartilage degradation was increased in TSK mice compared to aged-matched control mice, but subchondral bone plate was not modified. Levels of activated SMAD2/3, the main TGFβ signalling pathway, was not modified in TSK mouse knee joints compared to littermate controls.

Discussion This study shows that Fibrillin-1 is decreased from the pericellular matrix during OA development in spontaneous and trauma-induced OA, suggesting growth factor bioavailability may be modified during this period. In addition, mutations in Fibrillin-1 in the TSK mouse lead to abnormal ossification and OA development, which may not be linked to SMAD signalling. These data suggest that Fibrillin-1 plays an important role in joint homeostasis and that abnormal expression or mutations can lead to OA development.

Allosteric inhibition of ADAMTS-5 by monoclonal antibodies

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Introduction ADAMTS-5 is a member of the ADAMTS family of metalloproteases and cleaves the core protein of aggrecan, a proteoglycan representing a major component of articular cartilage matrix. Loss of aggrecan occurs in osteoarthritis (OA) and studies with knock out mice indicated that ADAMTS-5 ablation protects from disease progression. We therefore aimed to isolate inhibitory antibodies of ADAMTS-5 in order to determine whether they could act as chondroprotective agents.

Materials and Methods Anti-ADAMTS-5 antibodies were isolated from a human phage display library and screened first for binding to immobilized ADAMTS-5 (ELISA) and subsequently for enzyme inhibition using both a synthetic peptide and aggrecan as substrates. Epitope mapping was performed by surface plasmon resonance (SPR) using domain deletion mutants of ADAMTS-5. The efficacy of anti-ADAMTS-5 antibodies to block aggrecan degradation was tested using cartilage explants from patients undergoing knee replacement for OA.

Results Among the isolated antibodies, 2D3 inhibited ADAMTS-5 activity against both the peptide substrate (IC_{50}: 2.5 nM) and aggrecan (IC_{50}: 90 nM), whereas 2B9 inhibited only cleavage of aggrecan (IC_{50}: 70 nM). SPR studies with domain deletion mutants of ADAMTS-5 indicated that 2D3 binds to the catalytic/disintegrin domains, whereas 2B9 binds to the spacer domain of the enzyme. Competition studies with the N-terminal domain of the endogenous inhibitor of ADAMTS-5 (N-TIMP-3) showed that the binding site of N-TIMP3 does not overlap with that of 2B9 or 2D3. However, the binding of full-length TIMP-3 competed with the binding of 2D3, but not of 2B9. Both antibodies were able to inhibit aggrecan degradation in cartilage explants from OA patients.

Discussion The anti-ADAMTS-5 antibodies described here bind outside the active site cleft, in particular to the disintegrin (2D3) and spacer (2B9) domains. These antibodies represent a promising therapeutic approach as well as a useful tool to investigate the role of ancillary domains in aggrecanolytic activity of ADAMTS-5.

Demethylation of a crucial NF-*K*B enhancer element orchestrates iNOS induction in osteoarthritis via cell cycle regulation

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[1]*Institute of Developmental Sciences, Southampton General Hospital, Southampton SO16 6YD, UK*

Introduction Osteoarthritis (OA) is a complex disease of the joint, characterized by progressive degradation of the cartilage matrix by aggrecanases and collagenases. Nitric oxide (NO), the product of inducible nitric oxide synthase (iNOS), not only suppresses the synthesis of cartilage matrix, but also increases expression of proteases in OA. DNA methylation is an epigenetic mechanism implicated in the induction of iNOS in OA. We have examined the methylation profile of the NF-KB enhancer region at −5.8 kb of this gene, important in OA to determine the role in iNOS induction of OA.

Materials and Methods Femoral heads were obtained after joint replacement surgery due to OA or following a fracture of the neck of femur. The chondrocytic cell line C28/I2 was employed for transfection experiments.

Genomic DNA and RNA were extracted simultaneously using the Qiagen AllPrep DNA/RNA mini kit. DNA was modified with EZ DNA Methylation-Gold™ Kit. Pyrosequencing was used to analyse the status of methylation of the CpG localised inside the enhancer element −5.8 kb of iNOS.

The enhancer element (−5.8 kb) and promoter regions of iNOS were PCR-amplified. Each construct was cloned in the pCpG-Luc backbone and treated with a CpG DNA methyltransferase. C28/I2 cells were transfected using FuGENE for 48 h then washed and fixed with 70% ethanol, Guava analysis was carried out and FlowJo software employed to characterize the different cell cycle subpopulations. Cell viability assay was carried out using MTT protocol.

Results *In vitro* de-methylation of the CpG sites localised at −5.8 kb using 5-azadeoxycytidine resulted in lower DNA percentage in control chondrocytes, which correlated with higher levels of iNOS expression. *In vitro* methylation of the NF-κB enhancer region at −5.8 kb showed high levels of apoptosis and G0/G1 arrest.
Discussion The NF-KB enhancer element localised at −5.8 kb upstream of the transcription start site is important for iNOS induction. Loss of methylation of this region correlated with lower levels of apoptosis, enhanced proliferation and increased cells at G2/M phase. The loss of methylation and consequent higher iNOS expression observed therefore, could be transmitted to daughter cells during cell division and, critically, contribute to OA pathology.

Lithium chloride triggers primary cilia elongation and inhibits hedgehog signalling in articular chondrocytes

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Introduction In osteoarthritis (OA), the hedgehog signalling pathway is activated and promotes chondrocyte hypertrophy and matrix catabolism through the upregulation of ADAM-TS-5 and MMP-13. Inhibiting this pathway reduces cartilage degradation in surgical models of OA. Hedgehog signalling requires the primary cilium, a microtubule-based organelle present on the majority of chondrocytes. The trafficking of hedgehog signalling proteins through the ciliary compartment is essential for pathway regulation. Recent studies indicate that lithium chloride (LiCl) is chondroprotective largely due to its anti-inflammatory properties. LiCl modulates cilia structure in numerous cell types. We hypothesise that LiCl may also affect cilia-mediated signalling pathways in chondrocytes, like hedgehog, through the regulation of ciliary structure.
Materials and Methods Articular chondrocytes were treated with 0–50 mM LiCl for up to 24 h. Immunocytochemistry and confocal imaging were used to measure primary cilia length and prevalence. Activation of the hedgehog signalling pathway in response to recombinant Indian hedgehog (r-Ihh) was quantified using real-time PCR for GLI1 and PTCH1.
Results LiCl induced dose dependent primary cilia elongation, such that mean cilia length was increased by 95% in response to 50 mM LiCl. Cilia elongation was rapid, with the majority of growth occurring within the first hour resulting in an increased proportion of cilia with bulbous tips. Following r-Ihh treatment, the expression of GLI1 and PTCH1 was significantly increased by 5.22 and 4.23-fold respectively indicative of pathway activation. Co-treatment with LiCl inhibited this response in a dose dependent manner such that 50 mM LiCl completely abolished pathway activation.
Discussion These data show that LiCl stimulates rapid, dose dependent cilia elongation in primary articular chondrocytes and inhibits hedgehog signalling. Recent studies show that the modulation of ciliary structure can affect the organisation of proteins at the distal tip of the ciliary compartment resulting and disrupts ligand-mediated hedgehog signalling. We therefore propose that pathway inhibition may be linked to the effects of LiCl on cilia structure, future studies will investigate this by examining the effects of LiCl on the localisation of Kif7 and IFT81 at the ciliary tip. This study highlights the potential for targeting the ciliary structure as a novel therapeutic approach to modulate hedgehog signalling and matrix catabolism in OA.

Direct evidence linking DNA methylation status to IL8 expression in osteoarthritis

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Introduction IL-8 is a chemokine produced by human osteoarthritic chondrocytes, which contribute to the pathophysiology of osteoarthritis. In previous studies, it was found that sequence spanning nucleotides −1 to −133 in the *IL8* proximal promoter is essential and sufficient for transcriptional regulation. Interestingly, this region contains three CpG sites and they are located close to the binding sites for NF-κB, AP-1 and C/EBP. However, little is known about the epigenetic mechanisms leading to the increased *IL8* expression.
Materials and Methods Human chondrocytes were isolated from the articular cartilage of femoral heads obtained from patients with femoral neck fracture (control, n = 15) or osteoarthritis (OA, n = 15). *IL8* expression and percentage CpG methylation in human chondrocytes were quantified by qRT-PCR and pyrosequencing to compare OA patients with osteoporotic controls. Primary human chondrocytes were cultured for 5 weeks in two groups: without treatment (control culture); and IL-1β combined with oncostatin M (IL-1β culture). The effect of CpG methylation on IL8 promoter activity was determined using a CpG-free vector; co-transfections with expression vectors encoding NF-κB, AP-1 and C/EBP were carried out to analyse *IL8* promoter activities in response to changes in the methylation status.
Results *IL8* expression in OA patients was 37-fold higher than in controls; three CpG sites of the IL8 promoter were demethylated in OA patients. A multiple regression analysis revealed that percentage methylation of the CpG site located at ~116-bp was the strongest predictor of *IL8* expression. IL8 expression was increased in IL-1β culture with decreased percentage CpG methylation. *In vitro* DNA methylation decreased IL8 promoter basal activity. NF-κB, AP-1 and C/EBP strongly enhanced IL8 promoter activity and

DNA methylation inhibited the effects of these transcriptional factors.
Discussion The present study demonstrates the key role of DNA methylation status on the expression of *IL8* in human chondrocytes. A quantitative relationship between percentage methylation and gene expression was observed in clinical samples. These data provide evidence linking the activation of IL-8, DNA demethylation and induction of the OA process with important therapeutic implications for OA treatment.

Design of a TIMP-3 mutant with an increased half-life for improved protection against cartilage breakdown

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Introduction Osteoarthritis is a joint disease characterised by degradation of cartilage extracellular matrix by matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs). Tissue inhibitor of metalloproteinase 3 (TIMP-3) has been shown to block cartilage degradation by inhibiting these enzymes. Work in our laboratory has shown that TIMP-3 is endocytosed by HTB94 chondrosarcoma cells and chondrocytes via the endocytic receptor LDL receptor-related protein-1 (LRP-1) reducing its *in vivo* half-life and its chondroprotective effect. We seek to engineer a mutant of TIMP-3 that does not bind to LRP-1 and that hence, has an increased half-life and chondroprotective effect.
Materials and Methods Several LRP-1 ligands have been shown to bind to the receptor via 2 lysine residues separated by 21 Å. We used *in silico* molecular modelling to identify those residues in TIMP-3 satisfying the binding motif criteria and then performed site-directed mutagenesis to mutate these lysine residues to alanine. We have now generated 22 mutants and expressed these in HEK-293 cells. We followed the endocytosis of the mutants over time by HTB94 chondrosarcoma cells by immunoblotting.
Results In keeping with other studies of LRP-1 ligand binding, we found that mutation of 2 lysine residues has a greater effect on the rate of endocytosis than mutation of a single lysine. To date, 4 of the double mutants have demonstrated 50% or more reduction in the rate of endocytosis by HTB94 chondrosarcoma cells compared to wild type TIMP-3. We have now generated stable HEK 293 cell lines for these mutants and will purify the proteins and evaluate their binding to LRP-1 by ELISA.
Discussion We have successfully engineered TIMP-3 mutants with increased half-life in cell culture, indicating that TIMP-3 shares the same LRP-1-binding motif as has been described for other LRP-1 ligands. We will confirm that the mutants retain their ability to inhibit prototypic metalloproteinases and aggrecanases *in vitro*. We will then assess the protective activity of the mutants in cartilage by adding them to IL-1-stimulated human and porcine cartilage explants and quantifying aggrecan release.

Investigating microRNA–target gene interactions in skeletal muscle during chick development

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Introduction MicroRNAs (miRNAs), short, non-coding RNAs, which act post-transcriptionally to regulate gene expression, are of widespread significance and have been implicated in many biological processes during development and disease, including muscle disease.

The myomiRs are miRNAs highly enriched in striated muscles, including skeletal muscles in developing somites. Recent advances in sequencing technology and bioinformatics led to the identification of a large number of miRNAs expressed in somites. For many of these miRNAs specific roles, in particular during myogenesis, have not yet been determined.

We aim to better understand interactions between miR-128 and one of its predicted targets, Eya4, specifically during skeletal muscle development in chick.
Materials and Methods To determine miRNA expression patterns, chick embryos were collected at different stages of development, fixed and subjected to in situ hybridisation using Locked Nucleic Acid (LNA)-containing probes. Putative miRNA targets were identified using miRBase and TargetScan databases and GO term analysis (DAVID bioinformatic resource) allowed short-listing of target genes expressed in skeletal muscle.

For selected genes coding region fragments were cloned, RNA probes were synthesised and expression patterns determined. To generate sensor constructs, 3′-untranslated regions (UTR) were cloned downstream of the luciferase reporter gene, mutants were generated, and both constructs were used in luciferase assays.
Results LNA in situ hybridisation revealed that miR-128 is expressed in the myotome but also in other tissues depending on the stage of development. RNA in situ hybridisation for Eya4 indicated a strong expression in the myotome. Luciferase assays showed that miR-128 can interact with Eya4 3′UTR leading to a decrease in luciferase activity, which is significantly rescued by mutating the miR-128 target site.
Discussion The results show that miR-128 and Eya4 are co-expressed in developing muscle; they interact through a miR-128 target site in the Eya4 3′UTR. Future works will investigate the role of miR-128/Eya4 interaction during muscle development using *in vitro* and *in vivo* approaches.

Roles of Roundabout and Slits in developing limb joint formation

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Introduction Roundabouts (Robos) and Slits act as axon guidance molecules in the retina, and in addition are known to have roles in survival and function of pancreatic beta cells, regulating neural tube developmentand controlling myoblast migration. They are also expressed in and around the joints where their function is unclear. Mutations in Slits, Robos and DSCAM have been linked to spinal disorders, such as scoliosis and kyphosis, in humans and animal models. Slits and Robos are clearly required for normal joint development and maintenance, though our understanding of their roles in these processes is rudimentary.
Materials and Methods Using in-situ hybridisation the normal expression patterns of Slits, Robos, DSCAM and F-spondin in the developing joints of mouse embryos have been investigated. To begin to address their function in joint development functional studies utilising transgenic mice (Slit1-/-; Slit2-/- and Slit1-/-Slit2-/-) were performed to deduce how Slit mutations affect joint development, focusing upon the limbs and spinal column. The chick embryo was also used to examine the effect of Slit and Robo protein misexpression upon limb joint development.
Results Slit misexpression in the developing chick limb causes limb and limb joint defects.

In mouse Slit mutants, limb joints seem to be normal and there is no evidence of up-regulation of Slits to compensate for Slit loss.
Discussion Ongoing work is aimed at studying the functions of Robo and Slit in the developing limb joints of mouse and chick embryos.

Competitive interactions between hedgehog and cytokine signalling: crosstalk at the chondrocyte primary cilium?

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Introduction Cytokine signalling through NFκB signalling plays well-described but complicated roles in the pathogenesis of arthritis and we have also recently shown that the chondrocyte primary cilium exerts influence over cytokine-induced NFκB signalling. Recently, it has been demonstrated that hedgehog (Hh) signalling also plays roles, albeit less clear, in not only joint development and maintenance of joint physiology, but also disease. The role of the primary cilium in modulating ligand-induced hedgehog signalling is well-documented including in chondrocytes. Using a cell and tissue approach we investigated the possibility of primary cilia-localised crosstalk between hedgehog and NFκB signalling in arthritic signalling.
Materials and Methods Using isolated bovine or human primary chondrocytes, the hedgehog pathway targets and interleukin targets were assessed by qPCR in interactive studies using co-treatments of indian hedgehog ligand (1 μg/ml) and cytokine interleukin-1β (10 ng/ml). The molecular events defining pathway interaction and temporal dynamics were assessed by western blot. Tissue (5 mm explant) co-treatment studies were followed by biochemistry, IHC and westerns defining the net catabolic profile for cartilage tissue explants of bovine origin.
Results Treatment of cells with IL-1β inhibited ihh-induced GLI1 and PTCH1 gene expression. In parallel ihh ligand inhibited IL-1β-induced ADAMTS5 gene expression. In tissue, modulation of hedgehog pathway by ligand and small molecule interventions altered tissue catabolism profile of cartilage tissue. In the absence of ihh, hedgehog inhibitor cyclopamine inhibited IL-1β induced tissue damage in a dose-dependent manner.
Discussion These studies provide preliminary evidence for an interaction of hedgehog and interleukin/NFκβ signalling in isolated primary cells and tissue which has important implications for cartilage pathology. This may underlie the apparently complicated roles for Hh in joint pathology. It also highlights the central role of cilium in transducing biological relevant signalling in chondrocytes and cartilage, supporting the concept of ciliotherapies.

The coordinated regulation of BAF60 variants by miR-1/206 and miR-133 clusters stabilises myogenic differentiation during embryogenesis

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Introduction Formation of skeletal muscle in developing embryos is a multi-step process that involves transcriptional activation of muscle-specific genes from the coordinated action of myogenic regulatory factors (MRFs) and chromatin-remodelling complexes. The BAF complex is involved in a variety of cellular functions; BAF60a, -b, and -c are structural subunits of the complex and their binding to the core ATPase, Brg1, is mutually exclusive. The importance of BAF60c in recruiting the core during myogenesis is recognized. However regulation of the BAF complex and the roles of BAF60a and -b regulating subunit composition during myogenesis remain unclear.
Materials and Methods To study the expression profiles of the BAF variants during chick somitogenesis, we performed immunohistochemistry, qPCR and CoIP against Brg1. To knockdown BAF60 variants we used morpholinos and for

gain-of-function we used expression constructs electroporated into somites and performed *in situ* hybridisation for myogenin, a marker for myogenic differentiation. To determine whether BAF60a and -b were targeted by miRNAs, sensor constructs were generated with 3′UTR fragments containing putative miRNA binding sites downstream of luciferase. Function of miRNAs was inhibited *in vivo* by injection of antagomirs into somites. Regulation of BAF60a and -b by miRNAs *in vivo* was determined by Western blotting, CoIP and qPCR after injection of antagomirs.

Results We show BAF60a, -b and -c expression during somite differentiation. We further show the concerted negative regulation of BAF60a and -b by the myomiRs, miR-133 and miR-1/206, specifically in the myotome. BAF60c knockdown or sustained expression of BAF60a and -b resulted in delayed embryonic myogenesis. Inhibition of miRNA activity led to increased BAF60a and -b levels and a concomitant switch in Brg1/BAF subunit composition and delayed myogenesis. This phenotype was mimicked by sustained expression of BAF60a or -b and rescued by BAF60a or -b knockdown.

Discussion In summary, we demonstrate that miR-1/206 and miR-133 affect the combinatorial assembly of the SWI/SNF complex, specifically by post-transcriptional regulation of BAF60a and -b expression levels during development, thereby cementing the commitment of myoblasts to the myogenic differentiation program.

Role of Angiopoietin-like 4 and its regulatory pathways in tendon vascularization

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Introduction Tendinopathy is often viewed as the result of failed or inadequate healing response through repetitive overuse. Previous authors have suggested there may be an association between pain and neurovascular changes resulting from tendon overuse in tendinopathy patients. Our previous study has shown that cyclic strain induces an array of angiogenic genes in human tendon cells which promote angiogenesis. Our recent data introduced a new mechanoresponse protein named Angiopoietin-like 4 (ANGPTL4) which regulate angiogenesis. In our study we characterized the function of ANGPTL4 protein in tendon and its regulatory pathways in response to mechanical stimuli.

Materials and Methods The angiogenic activity of ANGPTL4 was determined with endothelial cell tube formation assay. Immunohistochemistry and gene expression analysis were conducted with mouse patellar tendons which were harvested after injection with ANGPTL4 protein. By using a Flexcell Tension System, isolated tendon cells from human hamstring tendons were exposed to cyclic tension. ANGPTL4 concentration and TGF-β activity in conditioned media were determined with ELISA and dual-luciferase reporter assays respectively. TGF-β protein and its receptor antagonist, hypoxic chamber, HIF-1α stabilizer and inhibitor were used to evaluate the mechanisms regulating ANGPTL4 production. Human cuff rotator tendon biopsies were used for immunostaining with ANGPTL4, HIF-1α and CD-31 antibodies.

Results ANGPTL4 protein induces endothelial cells tube formation, and angiogenic markers in mouse patellar tendon. The protein also induces MMP3 expression in the mouse tendon. Our findings illustrate that cyclic strain induces expression and release of ANGPTL4 through increased activity of TGF-β and stabilizing HIF-1α. The data from immunostaining of human rotator tendon tissue indicate a correlation between the expression of ANGPTL4, HIF-1α and endothelial cell marker which support the role of HIF-1α in induction of ANGPTL4 followed by angiogenesis.

Discussion This is the first study that unravels the role of ANGPTL4 protein in tendon. It seems that HIF-1α and TGF-β pathways modulate the expression and release of ANGPTL4 in response to cyclic loading. Therefore these pathways might have a key role in regulation of neovascularization during overuse tendinopathy. Our future studies on animal models will specify the effects of ANGPTL4 in course of tendon healing and injury.

Defining the molecular targets for plant cysteine proteinases on parasitic nematode extracellular matrix

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Introduction Parasitic nematodes cause enormous public health, agricultural and economic problems worldwide, as pathogens of humans, livestock and crops. Their impact is increasing due to lack of full efficacy of current anthelmintics and development of resistance by the nematodes. We are developing plant cysteine proteinases (CPs) as alternatives to currently available anthelmintics. We have demonstrated antihelmintic potency of plant cysteine proteinases from papaya ("papain"), fig (ficin), and pineapple (bromelain) on gastrointestinal (GI) nematodes of mouse, sheep and pig, *in vivo*. The enzymes have a novel mechanism, digesting the protective extracellular matrix (outer cuticle) of the nematode leading to blistering followed by rupture and death. The nematode cuticle is composed of proteins such as collagens and cuticlins but the specific molecular target(s) of the proteinases have yet to be identified. For example, there are about 158 collagen genes and 8 cuticlins in

the C. elegans genome. The aim of this study is to identify the molecular target(s) and thereby define the mechanism of action of this new class of anthelmintics, taking a proteomic approach.
Materials and Methods The free-living nematode, Caenorhabditis elegans and murine GI nematode Heligmosomoides bakeri are our initial target organisms. The worms were washed and frozen at −20°C. Worms were visualized by light microscopy and SEM. The cuticles were isolated by washing in denaturing buffers. They were digested with papain and other homologous CPs. The supernatant was taken, run on SDS-PAGE and individual bands were analysed by LCMSMS.
Results By microscopic examination the cuticle remained intact following freeze-thawing and washing. Initial experiments have confirmed the suitability of our approach, with cuticle globin, paramyosin and actin-related proteins identified in papain-digested cuticle supernatants.
Discussion The identification of actin, paramyosin and cuticle globin - a protein involved in the transport of oxygen across cells underlying the cuticle, is an indicator of CPs digestion of the cuticle; however, structural cuticle proteins attacked by CPs have yet to be identified.

Identifying the mechanisms of tendon generation: the importance of mechanical and chemical factors at different stages of development

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Introduction Tendon damage leads to the formation of scar tissue that is prone to re-injury. Before the efficient application of stem cell-based regenerative therapies, the processes regulating tenocyte differentiation should first be better understood. We previously demonstrated that equine embryonic stem cells (ESCs) undergo tenocyte differentiation in response to 3D culture and/or TGF-β3. We hypothesise that these signalling mechanisms are conserved in pluripotent cells and that they act through the transcription factor scleraxis.
Materials and Methods Equine induced pluripotent stem cells (iPSCs) were cultured in 2D in the presence or absence of TGF-β3. Tendon-associated gene and protein expression were measured and levels compared to those produced by ESCs cultured under the same conditions. Foetal and adult tenocytes expressing a short hairpin RNA (shRNA) against scleraxis (shSCX) or a non-target scrambled sequence (N-T) were generated through stable transfection. Scleraxis knock-down was measured and cell survival and ability to remodel a 3D collagen gel was determined.
Results We demonstrated that TGF-β3 promotes tendon differentiation of both equine iPSCs and ESCs in 2D culture. However, expression of tendon-associated genes is detected earlier in differentiating ESCs. We successfully established a shRNA against equine scleraxis, which knocked-down scleraxis gene and protein expression to undetectable levels. Scleraxis knock-down in adult tenocytes has no effect on their survival or ability to remodel a 3D collagen gel. However, while scleraxis knock-down in foetal tenocytes has no effect on their growth in 2D, it significantly reduces their survival in 3D and blocks their ability to remodel a collagen gel entirely.
Discussion We have demonstrated that TGF-β3 signalling drives tendon differentiation in equine pluripotent stem cells in 2D culture but ESCs appear to more readily differentiate down this lineage than iPSCs. Further work will compare the effect of 3D culture on tenocyte differentiation in these cells. Scleraxis knock-down has a significant effect on foetal tenocytes in 3D culture, suggesting that it plays an important role during the early stages of tenocyte differentiation. We anticipate that using scleraxis shRNA in pluripotent stem cells in 3D culture will allow us to further understand tendon developmental and reparative pathways.

MicroRNA regulation of chondrogenesis in human embryonic stem cells

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Introduction There is a huge unmet clinical need to treat articular cartilage defects in the UK with 140,000 knee and hip replacements being performed each year by the NHS. Embryonic stem cells (ESCs) offer a promising alternative therapeutic approach, potentially providing an unlimited source of chondrocytes capable of regenerating the damaged cartilage. Our lab has produced a chemically defined protocol for the generation of chondrocytes with over 95% of final cells expressing SOX9. However the chondrocytes produced lack the expression of extracellular matrix components secreted by mature chondrocytes. We aim to overcome this limitation by manipulating small non-coding RNAs know as microRNAs (miRNAs). Many miRNAs have been shown to play an important role in regulating chondrogenesis. This research aims to identify key miRNAs involved in chondrogenesis and through manipulation of these miRNAs enhance the efficiency of chondrocyte generation from ESCs.
Materials and Methods Human ESCs were differentiated into chondrocytes using the direct differentiation protocol developed by our lab [1]. At each stage of the protocol small RNA samples were extracted from the cells and sequenced using Illumina deep sequencing technology.
Results Deep sequencing of small RNAs at different stages of hESC-chondrogenesis has revealed several differentially expressed miRNAs throughout chondrogenesis including previously identified chondrogenic miRNAs with the most highly upregulated miRNAs being transcribed from Hox genes. MicroRNAs have also been identified in small extra-

cellular vesicles, known as exosomes, which are secreted during the protocol potentially providing a novel mechanism for cell-cell communication during chondrogenesis.
Discussion This research shows miRNAs are highly regulated throughout chondrogenesis and may lead to the discovery of novel miRNAs essential to chondrogenesis. In future experiments the functional properties of known and novel chondrogenic miRNAs will be examined by manipulating their expression using oligonucleotide mimics or inhibitors with the aim of improving chondrogenic differentiation of ESCs.

The role of the somites and notochord in vertebral column development

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Introduction Metameric patterning of the vertebrate trunk is established in the embryo upon segmentation of the paraxial mesoderm into epithelial balls of cells known as somites. These contain the precursors of the muscles and vertebral column in two distinct populations: the dermomyotome and sclerotome. In the adult, a muscle must insert into two successive vertebrae in order for the spine to bend. Therefore, the precursor populations must shift by half a segment with respect to one another during development. Intrinsic rostrocaudal patterning of the somite is known to be important in this process, but questions still remain regarding the relationship between somite and vertebral boundaries along the axis, and whether signals external from the somite are involved in this process. An obvious candidate for the source of external signals is the notochord.
Materials and Methods DiI and DiO were used to trace the fate of somites along the axis. The role of the notochord in patterning the sclerotome was tested by grafting an ectopic quail notochord adjacent to the somites of a chick host. In-situ hybridisation for sclerotome markers, labelling and time-lapse filming was used to reveal how the grafted notochord affects the patterning of vertebral precursors.
Results Our fate map reveals subtle regional differences in the relationship between somitic and vertebral boundaries. Grafting of a quail notochord adjacent to the somites of a chick host causes ectopic vertebral precursors to form around the graft, patterned in a more compact segmentation than the host. We test two alternative hypotheses to account for this: secretion of a uniform attractant by the notochord, which attracts host sclerotome and compresses segments as they move towards it, or that the notochord contains intrinsic segmental information for patterning somites, perhaps in the matrix. We show that segmentation of this ectopic sclerotome is the same regardless of the region from which the notochord graft was derived, and demonstrates an attraction of the somites towards an ectopic notochord in cultured embryos.
Discussion Our results demonstrate an important role for the notochord in positioning of the sclerotome at the midline by a powerful attractant, which has the potential to influence vertebral segmentation.

The thermodynamic identification of glucosepane cross-linking in type I collagen – an all atom molecular dynamics study

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Introduction The functionality of the musculoskeletal system is believed to be jeopardised by glycation and the accumulation of advanced glycation end products (AGEs). Some AGEs are generated by the non-enzymatic reaction of oligosaccharides with proteins in physiological systems. In collagen-rich tissues, such as tendons and ligaments, AGEs are believed to form covalent cross-links within and between collagen molecules, thereby changing the properties of the tissue. Glucosepane is by far the most abundant AGE cross-link in collagen with levels 100–1000 times higher than all currently known cross-links. However little is known about their site of formation.

Potential sites of glucosepane cross-linking in collagen were previously identified by determining the amount of time two amino acid residues were within a set distance of each other during a 100ns molecular dynamics simulation. However, the relative energetics of the potential cross-links of glucosepane were not considered. This study aims to identify specific sites involved in forming glucosepane cross-links within the tropo-collagen molecule, based on a relative energetics model using a proven fully atomistic molecular dynamics approach.
Materials and Methods A distance-based criterion search identifies lysine and arginine residues within 5 Angstrom of each other within a tropo-collagen. Fully atomistic Molecular Dynamics simulations exploiting the D-band periodicity to replicate the dense fibrillar environment are conducted under pseudo-physiological conditions. A site is a likely candidate for glucosepane formation if the total energy of the tropo-collagen molecule is lower in the presence of a bound glucosepane cross-link compared to an unbound glucose molecule.
Results Of the 24 positions identified based on the distance criteria, only six were found to be energetically favourable (exothermic binding enthalpies) compared to unbound glucose. Some of these favourable positions potentially have significant implications on the biological function of collagen, as they are within sites where key collagen-biomolecule and collagen-cell interactions occur.
Discussion Our model of a realistic 3-dimensional collagen fibril, has identified six likely sites for glucosepane formation within tropo-collagen. The positioning of these

sites is likely to have a significant effect on tissue function and integrity.

The role of fetal movement in prenatal hip joint morphogenesis

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Introduction Reduced or restricted fetal movements are thought to play a role in developmental dysplasia of the hip, where the hip joint is unstable or dislocated. Ralis and McKibbin describe the physiological changes during hip joint development, wherein the femoral head becomes more flat and the acetabulum more shallow over developmental time. In this study, we attempt to replicate the physiological trend in hip joint shape using a mechanobiological model, and investigate the hypothesis that reduced or restricted fetal movements could lead to hip instability.
Materials and Methods We developed a mechanobiological model of an idealised prenatal hip joint, based on our previously published model of joint shape morphogenesis. The model is composed of two opposing rudiments: the proximal femur and the acetabular region of the pelvis. In our previous work, we proposed a novel theory for the adaptation of growing cartilage to applied mechanical stimuli, in which growth and adaptation are stimulated by biological (chondrocyte density) and mechanical (hydrostatic stress) factors. Physiological changes in fetal movements and growth rate were simulated together with abnormal movement conditions, which consisted of reduced movements at the 1) early, 2) middle and 3) later stages of development.
Results Our model successfully replicates the physiological trends of acetabular and femoral head shape changes reported by Ralis and McKibbin. When a reduced movement was simulated at the early stage, the femoral head sphericity decreased and the acetabulum became shallower, resulting in a less stable joint. Reduced movements at the middle or later stages of development resulted in minimal joint shape changes and no loss in joint stability.
Discussion This research provides insights into the importance of mechanical forces due to prenatal movements for development of functional hip joints, and demonstrates that fetal movements, especially during early development, are extremely important for joint stability at birth. Reduced fetal movements may provide an explanation for the manifestation of congenital disorders such as DDH in the infantile hip.

Fat–Hippo signalling in bone development

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Introduction Fat4 and Dchs1 are protocadherins that act as a signalling pair. Fat, the Drosophila homologue of Fat4, regulates the Hippo and PCP pathway. There is evidence that the Fat-PCP and Fat-Hippo pathways are conserved in vertebrates, but it is unclear how well conserved they are. In humans, loss of function mutation of *FAT4* and *DCHS1* lead to Van Maldergem syndrome, characterised by craniofacial abnormalities and increased susceptibility to bone fracture amongst other defects. Yap/Taz, key components of the Hippo pathway, regulates osteoblast differentiation. Taz is necessary for osteoblast differentiation from mesenchymal stem cells, whilst Yap has been shown to inhibit Runx2, the osteoblast determination factor.
Materials and Methods MicroCT analysis of *Dchs1*$^{-/-}$ and *Fat4*$^{-/-}$ P0 mice was carried out. *Dchs1*$^{-/-}$ and *Fat4*$^{-/-}$ primary calvaria osteoblast cultures were analysed for alterations in differentiation by assessing alkaline phosphatase (ALP) activity. To determine if the Hippo pathway is deregulated, *Dchs1*$^{-/-}$ and *Fat4*$^{-/-}$ or wildtype osteoblast cultures were transfected with a generic Yap/Taz reporter plasmid. Rescue experiments were performed by transfecting osteoblasts with yap miRNA plasmids to reduce Yap expression or control plasmids and ALP activity was measured.
Results MicroCT analysis revealed bone defects in the craniofacial complex and appendicular skeleton in *Dchs1*$^{-/-}$ and *Fat4*$^{-/-}$ mutants. *In vitro* analysis showed a reduction in ALP activity of calvaria osteoblasts in the absence of Fat4/Dchs1, suggesting an intrinsic defect in osteogenic differentiation. Hippo reporter assay revealed that Yap/Taz activity is increased in *Fat4*$^{-/-}$ and *Dchs1*$^{-/-}$ calvaria and limb osteoblasts compared to wild type osteoblasts. Reducing Yap expression in *Fat4*$^{-/-}$ osteoblasts led to an increase in ALP activity compared to control.
Discussion The rescue of ALP activity in *Fat4*$^{-/-}$ osteoblasts suggests that increased Yap activity contributes towards the defect in osteoblast differentiation in *Fat4*$^{-/-}$ cultures. Together, these results showed that Fat4/Dchs1 signalling plays an important role in bone development, and that Yap acts downstream of Fat4 in the regulation of osteoblast differentiation.

The thermodynamic characterisation of glucosepane cross-linking in the extra cellular matrix: a density functional theory study

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Introduction Collagen, in the presence of oligosaccharides, can undergo a non-enzymatic glycation reaction known as the Millard reaction. Lysine and arginine side chains situated along the tropocollagen triple helix, covalently cross-link with a D-glucose molecules to yield glucosepane, an advanced glycation end product (AGE). The low proteolytic rate of collagen leaves collagen rich tissues susceptible to the build-up of AGEs. It is thought that high concentrations of AGEs are responsible for diseases such as atherosclerosis, renal insufficiency and further complications of diabetes. The glycation reaction mechanism by Biemel et al. has yet to be fully characterised thermodynamically using electron structure methods. Nasiri et al. proposed an alternative thermodynamically viable formation of glucosepane. However, their electron structure calculations described a reaction pathway of the reactants α-oxoaldehydes, methyl amine and methyl guanidine as substitutes for D-glucose, lysine and arginine, respectively. We use Density Functional Theory (DFT) calculations on complete lysine and arginine amino acids and a D-glucose molecule to thermodynamically characterise the reaction pathway proposed by Biemel et al. We also explore an alternative reaction pathway where arginine is present before lysine.
Materials and Methods All geometry/transition state optimisation was performed using the wb97xd functional, which includes empirical dispersion and better treatment of hydrogen bonds and van der Waals interactions. Initial optimisation calculations used the 3-21g basis set. Extension of the basis set to 6-31+g(d,p) was used for further optimisation. Vibrational analyses were performed on all optimised structures using the same functional and basis set. Intrinsic reaction pathway calculations confirmed that all transition states yielded the expected reactant and product.
Results The reaction pathway proposed by Biemel et al. was characterised using DFT. The cascade of reactions point towards a thermodynamically viable process, and the final formation of glucosepane was energetically more favourable than the reactants. Many of the transition states were found to reside on a flat potential energy surface, indicative of the long reaction times observed during experimentation.
Discussion The thermodynamic characterisation of Biemel's pathway provides a framework for comparison to future exploration of alternative AGE reaction mechanisms.

Low oxygen tension and 3D differentiation of human embryonic stem cells toward chondrocytes

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Introduction Articular cartilage injury is of great concern in orthopaedic medical treatment especially in young patients. A number of strategies to deal with this problem have been developed including cell-based therapy methods. Human embryonic stem cells (hESCs) are pluripotent and can differentiate into all cell lineages: both a benefit and a disadvantage for targeted differentiation to a desired cell type. We have developed an efficient protocol to generate chondrocytes from hESCs; however, the cells generated are immature. As it is known that the oxygen concentration in a number of human stem cell niches and in the articular joint are generally much lower than ambient, we are investigating improved conditions for stem cell growth and chondrogenic differentiation using lowered oxygen tension and in 3D.
Materials and Methods hESCs were cultured and expanded on mitotically inactivated feeder cells and subsequently transferred to feeder-free culture. After several passages to provide hESCs without residual feeder cells, a refined directed 14 days differentiation protocol for chondrogenesis was set up to generate chondrocytes. We tested preconditioning of hESCs in 3 sub-ambient O_2 levels, then directed differentiation of hESCs toward chondrocytes in parallel under ambient oxygen level (20%) and hypoxic oxygen level (3%). In addition to improve the formation of chondrogenic cells, we also investigate the effect of culturing hESCs and chondrogenic differentiation in the several 3D formats under ambient oxygen level (20%). The cells were characterised by qRT-PCR using a range of chondrocyte-selective marker genes and immunofluorescence for SOX9 staining. Glycosaminoglycan (chondroitin sulphate) content in the aggregates of chondroprogenitors was also detected by Safranin O staining.
Results Our results show that hESCs show a modest favourable response to directed differentiation into chondroprogenitors under lowered oxygen (3%) compared to "normoxic" conditions (20%) after preconditioning. This was demonstrated by some increased expression of chondrocyte marker genes, immunofluorescence and glycosaminoglycan staining. Three dimentional culture also gave a modest increase in chondrogenic gene expression.
Discussion Understanding the mechanisms driven by hypoxia, the role of hypoxia inducible factors and 2D versus 3D culture will inform stem cell protocols for generating chondrocytes for clinical applications for cartilage repair.

Defining the role of Shh signalling in satellite cell-mediated myogenesis

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Introduction Regeneration of adult skeletal muscles largely depends on the existence of tissue-specific stem cells known as satellite cells. These cells are highly regulated by extrinsic cues, including well-characterised embryonic signalling cascades. Sonic Hedgehog (Shh) signalling has pleiotropic functions in the control of patterning, cell proliferation and differentiation during early development. Specifically, we have previously shown that Shh signalling is required during skeletal myogenesis in the embryo by activating muscle-specific transcription factors and genes encoding basement membrane proteins. In the adult, the precise role of Shh signalling during satellite cell-mediated myogenesis remains poorly understood.
Materials and Methods Here we have used a myofibre culture system, which allow the study of satellite cells in their quiescent state as well as their activation upon culture while maintaining their association with the fibre and the basal lamina, which have essential role in preserving the stemness of satellite cells.
Results Using this system, we have demonstrated that genes encoding Shh pathway components are induced during adult myogenesis and that activated mouse satellite cells respond to Shh signals. Using Shh signalling agonists and inhibitors, we have begun dissecting the stages of adult myogenesis requiring Shh signaling. Taken together, our results indicate a role for Shh signalling in the control of satellite cell decision to exit the cell cycle and differentiate during adult myogenesis.
Discussion These findings are important as they provide us with a better understanding of the extra-cellular cues that control satellite cell activity. This knowledge will also be invaluable to design novel protocols for maintaining satellite cells *in vitro* prior to their use for cellular therapies to treat muscle disorders.

Effect of in utero vitamin D depletion on offspring skeletal development

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Introduction The impact of vitamin D deficiency during pregnancy on future offspring bone health remains undetermined, despite recognised links to post-natal bone disease. Understanding whether vitamin D status is influential within the developmental origins of bone disease is important when considering the scale of deficiency status worldwide. We hypothesise that *in utero* vitamin D deficiency results in compromised bone cell behaviour, thereby affecting the bone quality across the different levels of bone structure.
Materials and Methods In this study, a rodent model of complete in-utero vitamin D deficiency was utilised to investigate mRNA expression of femora samples from offspring at 140 days of age. Additional physical analysis of bone quality was also conducted through μCT to determine mineral density and structural health, reference point indentation for micro-mechanical behaviour and three-point bend testing for fracture toughness and bone strength measurements.
Results Examination of osteoblast mRNA provided no significant differences in *Runx2*, *Col1*, *Opn* and *Ocn* transcript levels between control and deplete groups for either gender ($n = 8$). μCT analysis yielded no differences in bone mineral density or morphological parameters regardless of maternal diet background. Mechanically, no differences were found between the dietary conditions when comparing micro-indentation at multiple femur locations, strength values obtained using three point bending tests and fracture toughness tests.
Discussion No consistent and observable differences were found in bone health of 140 day-old animals, despite differences in vitamin D status *in utero*. Recent studies have shown no links between vitamin D levels in pregnancy and childhood bone health in humans, which are supported by these findings. This indicates, at the one time point examined (140 days):

a) No link is present between bone health and vitamin D depletion or that.

b) Compensatory mechanisms are active to minimise the vitamin D depletion, either during the *in utero* or post-natal developmental phases in this rodent model. Further longitudinal studies are required to delineate if any compensatory mechanisms are active at the cellular level and whether the effects of vitamin D deficiency can be viewed at other ages.

The role of Histone Deacetylase 3 in chondrogenesis and osteoarthritis

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Introduction Histone deacetylases (HDACs) regulate the acetylation pattern of chromatin to control gene expression and determine cell differentiation and mammalian development. Previous findings suggest that the TSA HDAC pan-inhibitor can abrogate *MMP1* and *MMP13* expression in human articular chondrocytes (HACs) and inhibit cartilage degradation in bovine nasal cartilage explants and also protect against articular cartilage damage *in vivo*. Our aim is to examine the ability of a range of HDAC inhibitors and a HDAC3- targeting small interfering RNA (siRNA) to protect against cartilage degeneration *in vivo* and *in vitro* and

to investigate the role of HDAC3 in chondrocyte differentiation. Our goal is to achieve a better understanding of the potential role of HDAC3 in the development and/or progression of osteoarthritis (OA).
Materials and Methods Chemical inhibitors against all HDACs (TSA), class I HDACs (MS-275) and a selective HDAC3 inhibitor (Apicidin) as well as HDAC3 RNAi were used in a chondrosarcoma cell line (SW1353) and the expression of genes critical for cartilage erosion was subsequently measured. Human mesenchymal stem cells (MSCs) were also treated with the siRNA against HDAC3 to define its role in chondrogenesis.
Results After inhibition or down-regulation of HDAC3 in the SW1353 cells, the expression of the cytokine-induced major collagenases *MMP1* and *MMP13* is significantly decreased. During chondrogenesis, depletion of HDAC3 resulted in a significant increase in the expression of anabolic genes that encode for extracellular matrix components, for example, *ACAN* and *COL2A1*.
Discussion These results indicate a potential role for HDAC3 in the regulation of the expression of both anabolic and catabolic chondrocyte genes, and in the progression of cartilage degeneration in OA. Future directions of this study include the use of a surgically induced OA mouse model to define the role of HDAC3 *in vivo* in cartilage. Our results will improve our understanding of the role that HDAC3 plays in OA and thereby identify where best to target for the development of new treatments, in order to treat the disease more effectively.

Glucosepane: investigating formation and structure in tendon collagen

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Introduction Glucosepane is an advanced glycation end-product (AGE) crosslink derived from glucose, lysine and arginine. Glycation comprises a series of non-enzymatic reactions between reducing sugars and proteins; the products can form irreversible covalent crosslinks that accumulate in long-lived proteins such as tendon type I collagen. Ageing is recognized as an important risk factor for tendinopathies, although the mechanism is not clear. Our previous work has shown an accumulation of partially degraded collagen in tendon suggesting that accumulation of AGE crosslinks and collagen resistance to turnover may be key in age-related decline of function. Pentosidine, another AGE crosslink, increases significantly with increasing age in tendon, although levels are low. Glucosepane has been found in other tissues at much higher levels. The aim of this study was to elucidate the structure for glucosepane using an experimental approach and to determine whether glucosepane forms in tendon tissue.
Materials and Methods *In vitro* synthesis of the Glucosepane was carried out by reacting D-glucose with free amino acids Boc-lysine and Boc-arginine, simulating pseudo- physiological conditions. Reaction products were analyzed using a 12 tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, which gives ultra-high resolving power and mass accuracy. Fragmentation of the reaction products (MS2) was performed by electron-induced dissociation (EID) and collisionally-activated dissociation (CAD) providing insight into glucosepane structure. The presence of glucosepane in Human Achilles tendon was evaluated using the glucosepane standard. The tissue was digested with protease enzymes and analysed by mass spectrometry.
Results The mass spectrum of the synthesised glucosepane showed a product with a mass of 629.35039 Da, which correlates with the proposed structure for glucosepane. Acid hydrolysis of glucosepane removed the Boc-protective groups, resulting in the observation of a new species with a mass of 429.24561 Da, as determined by external calibration. An identical mass of 429.24561 Da was also observed in the mass spectrum of the tendon tissue sample.
Discussion The results detect glucosepane in human tendon tissue for the first time. Glucosepane is likely to have a significant effect on the mechanical and biological properties of tendon collagen and may account for the age-related increase in tendon pathology.

Collagen Toolkits can resolve the specificity and determinants of binding to collagen of MMP-1 and MMP-13

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Introduction Collagens I and II are preferred substrates for MMP-13. MMP-13 recognizes the tropocollagen molecule, perturbs the triple helix, so that the active enzyme can cleave the collagen α-chains at the canonical site, Gly775-Leu776 in collagen II. The specific residues nearby that define MMP-13 binding depends remain unidentified.
Materials and Methods Wild-type and catalytically inactive MMP-13 were either purified from cell supernatant, or expressed in bacteria. We used our triple-helical peptide Collagen Toolkit libraries in solid-phase binding assays to study the recognition of collagen by MMP-13. We used MALDI-ToF mass spectrometry to investigate cleavage of Toolkit peptides by MMP-13.

Results MMP-13 did not bind Collagen Toolkit III, but bound two peptides of Toolkit II (a lower-affinity site in collagen D1, and a higher-affinity site in peptide II-44, the canonical site in collagen D4). Pro-MMP-13 bound well, like the active, cleaved enzyme, or its free hemopexin (Hpx) domain. Ala-scanning within the peptide revealed the residues that support adhesion of MMP-13. The susceptibility of dissolved Toolkit peptides to proteolysis was independent of the specific recognition of immobilized peptides by MMP-13; the enzyme cleaved several collagen peptides in solution.
Discussion MMP-1 required 2 residues in particular, P1′ and P10′ (both Leu). MMP-13 binding was strongly dependent on these two, but also on several other residues, including some distal to the S10′ site recently described for MMP1. This implies that MMP-13 Hpx is crucial to the recognition of collagen by MMP-13, and that it enjoys a closer relationship with collagen than the MMP-1 Hpx. We found novel sites where MMP-13 can clip peptides in solution, and since the loci involved were not identical with the pattern of binding, we suggest that it may be the consequence of attack upon single strands, where peptides begin to unfold at physiological temperature. Thus the binding of MMP1 and MMP-13 to collagen is not identical. The Hpx is more closely involved in the latter. Moreover, the ability of both pro and active forms of MMP-13 but not MMP-1 to bind our peptides suggests different dynamics within and between the collagenase domains in the two enzymes.

The tissue specific functions of the circadian clocks: implications in diseases and tissue regeneration

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Introduction Circadian (24 hourly) rhythms temporally coordinate our daily physiology, metabolism and behaviour. Disruptions to circadian clocks have been associated with a wide spectrum of human diseases. But how circadian rhythm regulates tissue-specific physiology and pathology is still largely unknown. The objectives are to compare the entrainment mechanisms and downstream targets of the circadian clocks in cartilage, tendon and mammary gland tissue, and to investigate their changes during development, ageing and in disease.
Materials and Methods Tissue explants were obtained from aged and young mice with the clock reporter PER2::luc, and real-time bioluminescence recording and imaging were used to characterize the clock properties. Time-series microarrays were performed on mouse tissues collected every 4 h over 48 h to identify rhythmic genes. Global or tissue-specific "clockless" mouse models were used to characterize the corresponding tissue pathologies.
Results Autonomous circadian clocks in mouse cartilage, tendon and mammary gland tissue were identified, which can be entrained by systemic factors such as body temperature rhythm, hormones or cellular microenvironment. PER2::luc circadian oscillations were significantly lower in amplitude in all three tissues from aged mice. The circadian transcriptome analysis revealed hundreds of rhythmic genes in each tissue studied, including genes involved in tissue homeostasis, cell survival, and the pathogenesis of corresponding tissue diseases. Interestingly, the majority of the rhythmic genes do not overlap between any two tissues studied. Global or conditional ablation of the molecular clock in mice led to various pathologies, such as degeneration of the articular cartilage, calcification of the tendon, and impaired stem cell renewal of the mammary gland. Work is underway to elucidate the initiation of the circadian timing event during chondrogenesis using human ES cells, and to test whether circadian rhythm plays a role in the maintenance of the differentiated phenotype.
Discussion These results reveal functional circadian clocks in the musculoskeletal system and the mammary gland, which rhythmically regulate key aspects of tissue homeostasis and pathology. We envisage a scenario where chronic circadian disruption or misalignment (e.g. during aging or shift work) may cause an internal desynchronization and a decline of various clocks, compromising tissue homeostasis and increasing susceptibility to diseases.

The miR-29 family in osteoarthritis

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Introduction MicroRNAs (miRNA) are short endogenous non-coding RNA molecules, negatively regulate gene expression. In osteoarthritis (OA), several genes necessary for cartilage homeostasis are aberrantly expressed, with a number of miRNAs implicated. The purpose of this study was to identify miRNAs involved across OA using both a murine model and human cartilage, and to define their function.
Materials and Methods Total RNA was purified from knee joints taken from mice at day 1, 3 and 7 post-DMM (destabilisation of the medial meniscus) surgery. Expression of miRNAs and mRNAs was analysed by microarray and validated by qRT-PCR. Expression was analysed by qRT-PCR in primary human chondrocytes stimulated with TGFβ1, IL-1β, LPS or Wnt3A; or after manipulation of SOX9. miR-29a/b1 promoter activity was measured using promoter-reporter constructs. Functional interaction between miR-29 and Smad, NFKβ and canonical Wnt signalling pathways was explored using luciferase reporters. Novel targets of the miR-29 family were characterized by gene expression pro-

files following transient transfection with miRNA mimic or inhibitor. Direct targets were confirmed by cloning the 3′UTR downstream of a luciferase reporter.
Results Differential miRNA expression in whole mouse joints post-DMM surgery increased over 7 days. Among the modulated miRNAs, the expression of miR-29b was regulated in the opposite direction to its potential targets. In end-stage human OA cartilage, the miR-29 family were also regulated. TGFβ1 and LPS decreased miR-29 family expression whilst IL-1β increased their expression. Functionally, the miRNA 29 family were negatively regulators of Smad, NFKB and canonical Wnt signalling. SOX9 directly represses the expression of miRNA 29a/b1 via binding to the promoter. Gain- and-loss-of-function experiments revealed regulation of known miR-29 target genes, an additional subset of TGFβ1-inducible genes and Wnt signalling pathway-related genes. Among these, ADAMTS6, ADAMTS14, ADAMTS17, ADAMTS19, FZD3, FZD5, DVL3, FRAT2 were validated as miR-29 family direct targets.
Discussion These data identify the miRNA 29 family early in the development of OA. They are regulated by a number of factors known to act in OA. They have functional impact on relevant signalling pathways with a number of direct targets identified. Future work will continue to dissect the role of the miR-29 family in OA.

Meox2 is necessary for axial and appendicular tendon development

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Introduction Tendon is a fibrous connective tissue that connects bone to muscle and bone to bone. Axial tendon progenitors arise from the syndetome, a somitic compartment, while limb tendons arise from lateral plate mesoderm. Tendon development is regulated by FGFs and TGFß2/3 signalling molecules. Tendon cells express scleraxis (Scx) and in Scx-/- mice tendon development is disrupted from E13.5. Other transcription factors: Mohawk, Sox9 and Egr1/2, are also important for later stages of tendon development. Meox2 is an essential regulator of limb myogenesis. Furthermore, at P0 Meox2-/- mutant mice tendons are brittle, pale and thin. We hypothesize that Meox2 is an essential regulator for embryo tendon development. My project aims to investigate when the tendon defect arises in Meox2-/- embryos and whether Meox2 acts intrinsically or extrinsically in tendon progenitor cells.
Materials and Methods Meox2-/- mice,Meox2-nLacZ mice, Scleraxis-GFP mice, Immunohistochemistry, In situ hybridization, Ex-vivo assay, In-vitro assay.
Results When we crossed ScxGFP mice with Meox2-/- mice, we found a strong decrease in ScxGFP levels in Meox2-/- limb and tail tendons at P0. In Meox2-/- embryos at E14.5, ScxGFP distribution in digit tendons was disrupted. In situ analysis showed reduced levels and domains of Scx transcripts as early as E12.5, preceding the defects in the Scx knockouts. Analysis of Meox2 protein and ScxGFP at E12.5 showed predominantly domains of either Meox2+/Scx- or Meox2-/Scx+ cells and few double positive Meox2+/Scx+ cells. By E13.5, all Meox2+ cells are expressed in mature tendon.
Discussion At E12.5, TGFß signalling is required for recruiting Scx+ cells at functional site and we hypothesize that TGFß signalling is also recruiting Meox2+ cells to tendon cell fate. Whether Meox2 is regulated by TGFß signals will be investigated by using TGFßR mutant embryos and ex-vivo limb bud culture assay. The fact that at E12.5 cells are predominantly Meox2+ or Scx+ will be investigated by lineage tracing using a Meox2-nLacZ allele to determine if Scx+ cells originate from a Meox2+ progenitor population.

Identifying diet-derived chondroprotective compounds in osteoarthritis

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Introduction Current pharmacological intervention for osteoarthritis (OA) is focused on inflammation and pain relief rather than addressing the degradation of articular cartilage. Such treatments typically have a single mode of action in a multifactorial disease. Compounds which can be derived from the habitual diet can be seen as an attractive alternative, since foods typically contain multiple bioactive compounds that can interact with multiple cellular pathways. The purpose of this study was to identify novel chondroprotective compounds found in the human diet.
Materials and Methods Since matrix metalloproteinase-13 (MMP-13) is considered a key collagen-degrading enzyme, inhibition of MMP13 expression, measured by qRT-PCR, was used as a surrogate marker of cartilage degradation. Ninety-six diet derived compounds were selected from a list of natural products based on (i) the edibility of the source; (ii) how common its source was in the human diet; (iii) whether the compound had previously been studied in chondrocytes. Compounds (at 10 μM) were screened in triplicate against basal expression and inhibition of interleukin-1 (IL-1)-induced expression of MMP13 in SW1353 chondrosarcoma cells and the C28/I2 transformed human chondrocyte cell line. The lead compounds from these screens were then assayed in three isolates of primary human articular chondrocytes for their impact on expression of MMP13, MMP1, ADAMTS4 and ADAMTS5. Compound toxicity was measured using lactate dehydrogenase release and FACS.
Results All compounds tested were non-toxic at 10 μM. Six compounds significantly reduced MMP13 expression in SW1353 cells, whilst eleven compounds significantly

reduced MMP13 expression in C28/I2 cells ($P < 0.05$ - $P < 0.0001$). Of the compounds assayed in primary human articular chondrocytes, five compounds significantly inhibited both MMP1 and MMP13 expression (apigenin, aloe emodin, emodin, luteolin and isoliquiritigenin). Apigenin significantly inhibited ADAMTS5 and aloe emodin significantly inhibited ADAMTS4 ($P < 0.05$ – $P < 0.01$). Apigenin, aloe, emodin and isoliquiritigenin showed dose-dependency across the 2.5–40 μM range.

Discussion This screen has identified a number of compounds which have the potential to be chondroprotective. Apigenin is a flavone found in various plants such as celery and swede; aloe emodin is a hydroxyanthraquinone found in aloe vera; isoliquiritigenin is a chalcone from licorice. These compounds will now be taken forwards for further analyses.

Deletion of SirT1 drives joint inflammation and suppresses anabolic chondrocyte gene expression

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Introduction Ageing is the primary risk factor associated with osteoarthritis (OA). The role of aging-related mechanisms in cartilage homeostasis is poorly understood. The class three histone deacetylase, Sirtuin 1 (SirT1) has been extensively shown to regulate lifespan in lower organisms and signalling pathways linked to mammalian aging. Deletion of SirT1 in a cartilage specific manner recently showed increased OA severity in mice. Here we examine the molecular role of SirT1 in chondrocyte and joint biology.

Materials and Methods Gene expression profiles were examined in the chondrocyte cell line (HTB-94) with the pharmacological inhibitor of SirT1 (EX-527; 100 nM), by siRNA knockdown, or by activation of SirT1 using (SRT1720; 500 nM). Conditional deletion of SirT1 in a pan tissue or cartilage specific manner was achieved by crossing $SirT1^{fl/fl}$ x ROSA Cre or $SirT1^{fl/fl}$ x Aggrecan Cre respectively.

Results SirT1 inhibition in HTB-94 cells by either EX-527 or siRNA reduced gene expression of COL2A1 ($P < 0.01$), Aggrecan ($P < 0.01$) and SOX-9 ($P < 0.01$) whilst not affecting MMP-13 or ADAMTS-5. Pharmacological activation of SirT1 stimulated the expression of COL2A1 ($P < 0.01$), Aggrecan ($P < 0.01$) and SOX-9 ($P < 0.01$). When whole joints from naïve SirT1 deficient animals were examined, many inflammatory genes were increased including IL-1β ($P < 0.01$), IL-6 ($P < 0.01$), CCR2 ($P < 0.001$), TSG-6 ($P < 0.01$), ADAMTS-1 ($P < 0.01$) and ADAMTS-4 ($P < 0.001$), MMP-13 ($P < 0.01$), NPY ($P < 0.0001$) and TIMP-1 ($P < 0.01$). Although COL2A1 and Aggrecan gene expressions were not regulated in these animals, they were down-regulated in joints from animals following cartilage specific deletion of SirT1. In the latter inflammatory gene expression was unchanged.

Discussion These results suggest that SirT1 has both anti-inflammatory effects globally as well as pro-anabolic cartilage effects, both of which may explain its chondroprotective role *in vivo*. Future directions will define the molecular response *in vivo* of SirT1 deletion following OA induction.

Umbilical cord mesenchymal stem cells suppress host rejection: the vital role of the glycocalyx

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Introduction Umbilical cord mesenchymal stem cells (UMSCs) have unique immunosuppressive properties enabling them to evade host rejection and making them valuable tools for cell therapy. We previously showed that human UMSCs survive xenograft transplantation and successfully correct the corneal clouding defects associated with the mouse model for the congenital metabolic disorder Mucopolysaccharidosis VII. However, the precise mechanism by which UMSCs suppress the immune system remains elusive.

Materials and Methods This study aimed to determine the key components involved in the ability of the UMSCs to modulate the inflammatory system, and to identify the inflammatory cells that are regulated by the UMSCs. Experiments were performed *in vivo* removing cell surface chondroitin sulphate or heparan sulphate prior to transplantation to determine if the glycosaminoglycans play a role in the immunoregulatory properties of UMSC. Moreover, *in vitro* co-culture assays were performed between UMSC and inflammatory cells to determine the mechanism by which UMSC modulate the immune system.

Results Our results show that human UMSCs transplanted into the mouse stroma 24 h after an alkali burn suppress the severe inflammatory response and enable the recovery of corneal transparency within 2 weeks. Furthermore, we demonstrated *in vitro* that UMSCs inhibit the adhesion and invasion of inflammatory cells, and also the polarization of M1 macrophages. UMSCs also induced the maturation of T-regulatory cells, and lead to inflammatory cell death. Moreover, UMSCs exposed to inflammatory cells synthesize a rich extracellular glycocalyx composed of the chondroitin sulphate proteoglycan versican bound to a heavy chain (HC) modified hyaluronan (HA) matrix (HC-HA), which contains TNFα-stimulated gene 6 (TSG6), the enzyme that transfers HCs to HA, and pentraxin-3, which further stabilizes the matrix.

Discussion Our results, both *in vivo* and *in vitro*, show that this glycocalyx confers the ability for UMSCs to survive the host immune system and to regulate the inflammatory cells.

The effects of adiopokines on canine cruciate ligament and meniscus

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Introduction Cranial cruciate ligament rupture (CCLR) is one of the most important causes of hind-limb lameness in dogs and morbidity in comparative species such as man. CCLR can lead to articular cartilage damage, meniscal tears, and the development of osteoarthritis (OA). Obesity is one of the risk factors associated with joint disease and OA. Adipocytes synthesise and release an array of chemical mediators named adipokines, and inflammatory mediators such as acute phase proteins, chemokines, and cytokines; these can result in inflammation and increased breakdown of body tissues. The purpose of this study is to assess gene expression for key adipokines, inflammatory mediators, and cartilage degradation markers in the stifle joint tissues from dogs with CCLR with different body condition scores, and to determine their relationship with disease activity.
Materials and Methods 25 ruptured CCLs and 13 torn meniscal samples were collected from dogs referred for surgical treatment of unilateral CCLR. Gene expression for key adipokines (adiponectin, leptin, visfatin), inflammatory cytokines (MCP-1, TNF-α, IL-6) and cartilage degradation biomarkers (AGC1, COL1, MMP-13) in CCL and meniscus samples were determined by quantitative reverse transcription polymerase chain reaction. The relationships among those genes was observed, and were compared statistically with clinical measures including age, weight, body condition score and severity of lameness.
Results There was no significant relationship between adipokines and inflammatory cytokines; however, we found positive correlation with adipokines and cartilage degradation biomarkers (adiponectin versus AGC1, $r^2 = 0.90$ and $P = 0.03$; leptin versus COL1, $r^2 = 0.62$ and $P = 0.004$; and visfatin versus MMP13, $r^2 = 0.34$ and $P = 0.02$). Moreover, positive correlations of age and MMP-13 ($r^2 = 0.14$ and $P = 0.04$), lameness score and AGC1 expression ($r^2 = 0.23$ and $P = 0.02$), and between TNF-α and body weight ($r^2 = 0.07$ and $P = 0.03$) were also identified. No significant relationship between body condition score and any gene expression had been found.
Discussion Our data suggest associations between gene expressions for key adipokines and cartilage matrix degradation, which can lead to the progression of OA. Moreover, the results indicate that expression of TNF-α may be associated with canine obesity and possibly involved in CCLR. Further work is required so as better to understand the role of adipose tissue in cruciate ligament pathology.

Trabecular adaptive response to loading in aged mice is restored by pre-exposure to supraphysiological loads

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Introduction Animals modify the shape and mass of their bones to accommodate both habitual and external mechanical forces in order to prevent fracture. The increased risk of fracture is very obvious in the aged, in whom a failure to achieve such mechano-adaptive structural modification leads to human suffering and enormous burden on the economy. Herein, we explore whether bone adaptation to loading in aged mice can be restored by the induction of background bone remodelling following brief exposure to supra-physiological loads.
Materials and Methods The right tibia of 20 month old mice (n = 10/group) were subjected to 40 cycles of 9N dynamic loading for 3 days/week for 2-weeks. In a separate group of 20 month old mice, the right tibia was subjected to two episodes of supra-physiological 11N loading, allowed to rest for 5 days, and then subjected to 2 weeks of 9N loading as described previously. MicroCT was used to assess the architectural changes in the cortical and trabecular bone.
Results Our data show that continuous 9N loading fails to provoke any significant changes in bone mass or architecture in either the trabecular or cortical bone compartment of the tibia in these aged mice. However, mice primed by receiving an earlier 11N bout of supra-physiological loading showed significantly increased trabecular BV/TV ($P < 0.05$) in right loaded tibia compared to non-loaded left. This load-induced increase in trabecular bone was also associated with significant increases in trabecular number ($P < 0.05$). In contrast, evaluation of cortical bone architecture at 37% and 50% (proximal) along the length of the tibia revealed a significant load-induced reduction in cortical thickness compared to controls ($P < 0.01$), which were matched by corresponding decreases in cortical tissue volume ($P < 0.05$).
Discussion Our findings suggest, that prior imposition of supra-physiological load, can act as a driver to restore and kick-start adaptive responses to loading in the skeletons of aged mice. This was however complicated by the observation that different rates of adaptive response appear to be elicited in cortical and trabecular bone. Exploring factors associated with this restoration of load-related mechano-adaptive behaviour in aged bones could identify possible targets to help alleviate the risk of fracture associated with frailty in the ageing skeleton.

Interleukin-1α stimulates ADAM17-mediated ectodomain shedding of LRP1 and reduces endocytosis of aggrecanases in articular cartilage

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Introduction We have recently demonstrated that major aggrecanases (ADAMTS-4 and ADAMTS-5) are rapidly endocytosed via low-density lipoprotein receptor-related protein 1 (LRP1) and degraded by chondrocytes in healthy cartilage, but this process is impaired in OA cartilage due to increased shedding of LRP1, which results in increased extracellular accumulation and activities of aggrecanases. Here, we investigated the effect of IL-1 on this endocytic pathway.
Materials and Methods Human primary and passaged chondrocytes were treated with 10 ng/ml of IL-1 in the presence or absence of protease inhibitors (AEBSF, E-64 or marimastat) or siRNAs targeting membrane-bound metalloproteinases. Cell surface protein and mRNA levels of LRP1 were measured by flow cytometry and real-time PCR, respectively. LRP1 protein shed from cell surface was measured by Western blotting. ADAM17 neutralising antibody was used to further confirm the sheddase activity.
Results IL-1 reduced the rate of ADAMTS-5 endocytosis without changing mRNA levels of LRP1. IL-1 markedly reduced the cell surface expression levels of LRP1 and increased the shedding of LRP1. Studies with protease inhibitors indicated that the major LRP1 sheddase(s) under these conditions is a metalloproteinase. Cells treated with siRNA-targeting ADAM17 or with an ADAM17 neutralising antibody showed markedly reduced IL-1-stimulated LRP1 shedding. Furthermore, we found that shed LRP1 forms complexes with ADAMTS-5, which makes the enzyme resistant to endocytosis.
Discussion Our study has shown that the reduced rate of ADAMTS-5 endocytosis upon IL-1 treatment is due to an increase in LRP1 shedding mediated by ADAM17. IL-1 treatment is thus likely to increase extracellular accumulation and activities of aggrecanases by reducing cell surface protein levels of LRP1 and by favouring accumulation of shed LRP1 in media. We propose that an increase in LRP1 shedding alters the trafficking of key aggrecanases and so contributes to slowly progressing chronic diseases associated with degradation of the extracellular matrix. (Supported by Arthritis Research UK project grant 20563).

High-throughput chondrogenic culture for pluripotent stem cells

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Introduction Cartilage is involved in skeletal development, covers the ends of long bones and is maintained by cells known as chondrocytes. The loss of cartilage function either due to mutations in specific genes or cartilage injury can cause skeletal dysplasia and osteoarthritis. Pluripotent human stem cells such as human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) can form all body tissues and have excellent potential for tissue regeneration, providing models for human disease, testing of pharmaceutical products and enhancing understanding of cartilage development. However, to exploit their full potential we need to produce reliable and reproducible protocols for generating differentiated chondrocytes at high-throughput and efficiency. The aim of this project is to develop a high-throughput protocol for hESC and iPSC differentiation to chondrocytes.
Materials and Methods A two stage approach (hESC->MSC->chondrocyte) was used in this project. Three alternative methods of hESC to MSC differentiation were tested; plating Hues1 hESCs directly on to gelatin, differentiation through embryoid bodies and spontaneous differentiation of hESC colonies. Chondrogenic potential of these cells was then assessed using a 96-well plate pellet culture system, modified from Penick *et al.* and compared to an already established protocol within the laboratory hESC, MSC and chondrogenic markers OCT4, CD146, CD166, CD73, CD44, SOX9, COL2A1 and ACAN were assessed using real-time PCR and normalised to GAPDH.
Results All three methods of inducing MSCs from ESCs gave a cell morphology similar to MSCs and increased expression of MSC markers CD146, CD166, CD73 and CD44. Pellet culture followed by real-time PCR for SOX9, COL2A1 and ACAN suggest the MSCs produced from spontaneous differentiation of ESC colonies have the greatest chondrogenic potential of the three methods tested.
Discussion With refinement, these hESC and iPSC chondrogenic protocols will enhance understanding of human development, provide models for human disease and a way of testing pharmaceutical products.

The effects of nivalenol on proteoglycan catabolism in bovine articular cartilage cultured in vitro

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Introduction Mycotoxin contamination is one of the risk factors for Kashin-Beck Disease (KBD), and nivalenol is a

member of the trichothecene family of mycotoxins contributing to onset of KBD. Our previous studies have illustrated an abnormal cartilage catabolism in the articular cartilage from KBD patients. However, it is still largely unknown whether mycotoxins, i.e. nivalenol, can induce proteoglycan catabolism, one of the key steps initializing articular cartilage degradation and KBD development.
Materials and Methods Bovine articular cartilage explants were obtained and incubated with or without 10 ng/ml IL-1beta. In addition, these samples were incubated with 0, 0.5 or 5.0 μg/ml nivalenol, respectively. sGAG content in cartilage explant was showed by toluidine blue staining. Sulphated glycosaminoglycan (sGAG) released into culture medium was determined using DMMB assay. Aggrecan catabolism was investigated using Western blotting.
Results Nivalenol in combination with IL-1beta did not alter sGAG staining pattern when compared with the samples treated with IL-1β alone. However, DMMB assay showed an increase in sGAG release in the combination groups of 0.5 μg/ml nivalenol and IL-1beta ($P < 0.05$), although 5.0 μg/ml nivalenol combined with IL-1beta decreased sGAG levels released in the culture medium ($P < 0.05$) when compared with the samples treated with IL-1beta alone. Nivalenol alone did not increase sGAG release ($P > 0.05$). Differently, western blotting results demonstrated that 0.5 μg/ml nivalenol alone increased aggrecan degradation in comparison with control samples ($P < 0.05$); the combination of 0.5 μg/ml nivalenol and IL-1beta significantly increased aggrecan degradation when compared with IL-1beta only samples ($P < 0.05$). However, 5 μg/ml nivalenol attenuated the aggrecan degradation induced by IL-1beta ($P > 0.05$).
Discussion Nivalenol induces proteoglycan loss from articular cartilage in a dose-dependent way. It contributes to the proteoglycan catabolism through enhancing the effects of IL-1beta. Nivalenol thereby play a role in the occurring and development of KBD by promoting matrix degradation.

Saikosaponin-d decreased chondroitin sulphated proteoglycan expression in rat hepatocellular carcinoma tissues

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Introduction Our previous studies have shown an increased expression in chondroitin sulphated glycosaminoglycan (CS-GAG) content in rat hepatocellular carcinoma (HCC), indicating that CS proteoglycan (PG) plays an important role in HCC occurring and development. Radix bupleuri is a classic Chinese herb for HCC treatment. Saikosaponin-d (SSd) is the most active monomer composition in radix bupleuri. However, its precise mechanism in anti-HCC functions is still largely unknown. This study is thereby to investigate whether SSd can affect CSPG expression during HCC prognosis.
Materials and Methods Forty Sprague-Dawley (SD) rats were divided into HCC and SSd groups. 0.2% (w/v) N-diethylnitrosamine was administered to all rats intragastrically. Meanwhile, 3% (w/v) SSd was administered to the rats in the SSd group. After 16 weeks from the initiation of experiment, all rats were killed and livers were collected. Histology was performed to demonstrate the onset of HCC and the content of sulphated glycosaminoglycan (sGAG). Immunohistochemical staining was performed to investigate the expression CS-GAG, and CSPG family members including aggrecan, versican, biglycan and decorin in HCC tissues.
Results H&E staining indicated that SSd significantly decreased malignant nodules on the surface of livers when compared with HCC groups ($P < 0.01$). Toluidine blue showed that SSd decreased sGAG content in tumor tissues ($P < 0.01$). Moreover, SSd inhibited CS-GAG expression in tumor tissues when compared with HCC group ($P < 0.01$). Further immunohistochemical staining results demonstrated that SSd reduced aggrecan, biglycan and decorin content in liver tissues when compared with that in HCC groups ($P < 0.01$). However, there was no alteration in versican expression in response to SSd treatment ($P > 0.05$).
Discussion For the first time, this study showed that SSd decreased the expression of CSPG family members including aggrecan, biglycan and decorin in HCC tissues, suggesting that SSd may play an anti-tumor role by inhibiting CSPG expression in HCC cells.

A hole in the head; development of cranial foramina in the chick embryo

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Introduction Cranial foramina are holes formed during development within the skull allowing entry and exit of blood vessels and nerves. Once formed they remain open, due to the vital structures they contain i.e. optic nerves, jugular vein and carotid artery. Understanding foramina development is essential as cranial malformations lead to the stenosis or complete closure of these structures, resulting in blindness, deafness, facial paralysis, raised intracranial pressure and lethalities. Currently no research has explored their development.
Materials and Methods Methods of investigation performed were histological and immunohistochemical techniques, in-situ hybridisation and embryonic techniques such as neural tube ablations.

Results Investigation of the blood vessel and nerve contents with smooth muscle actin (SMA) and acetylated anti-tubulin respectively found foramina in the base of the skull contain both. In addition the nerves innervate the blood vessel walls and the blood vessels are not immunoreactive for SMA.

Mesenchyme surrounding contents of foramina is initially dense and as development proceeds becomes sparser to clear and create overt cranial foramina with a perichondrium border. In situ hybridisation analysis with sox10 shows that the mesenchyme surrounding the contents of cranial foramina initially embarks on a skeletogenic fate but this is later switched off in this area specifically.

Hypoglossal ablations causing loss of the hypoglossal nerve result in absence of the hypoglossal foramina.

Investigations into the role of the c-type natriuretic pathway (CNP) which has known roles in endochondral ossification reveals the CNP ligand, GC-B receptor, NPR-C clearance receptor and PKGII are all expressed during cranial foramina development.

Discussion Cranial foramina formation involves a gradual "clearing" to make the foramina mesenchyme around foramina contents sparse as development proceeds. This mesenchyme also initially embarks on a skeletogenic fate, which is later halted to create "a zone of inhibition". Our results also reveal an important role of nerves in inhibiting chondrogenesis during foramina formation. The blood vessels in foramina arising in the base of the skull are part of a glomus body structure, which we hypothesize may play a baro-receptive role. In addition c-type natriuretic pathway signaling is present during cranial foramina development and may play an important role in their formation.

Long non-coding RNAs in chondrocyte development and cartilage

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Introduction Recent characterisation of the transcriptome has indicated the abundance of long non-coding RNAs (lncRNAs) derived from both intergenic and overlapping protein-coding gene regions. lncRNAs are expressed in a highly tissue-specific manner where they function in various aspects of cell biology. Although only a fraction have been functionally validated, lncRNAs have emerged as key regulators of gene expression, acting through diverse mechanisms such as the regulation of epigenetic modifications and by acting as scaffolds for protein complex formation at gene loci. The abundance of lncRNAs in chondrocyte development and cartilage has yet to be established. Thus the aims of this study are to illuminate the chondrocyte non-coding transcriptome and establish functions for lncRNAs in chondrocyte development and biology.

Materials and Methods RNA sequencing was performed on human articular cartilage RNA by Illumina Genome Analyzer IIx and reads aligned to the human genome. Bioconductor packages were used for normalization and to identify differentially expressed transcripts. The Tuxedo software suite was used to map novel transcripts. lncRNA expression was validated by real-time RT-PCR and rapid amplification of cDNA ends (RACE). Regulation of lncRNAs and the role of selected lncRNAs following targeted RNA interference (RNAi) was examined during differentiation of human mesenchymal stem cells (hMSCs) into chondrocytes, in comparison with adipocytes and osteoblasts.

Results We have identified the expression of 5000+ lncRNAs in cartilage tissue and identified a number of robustly expressed novel lncRNAs potentially specific to the chondrocyte phenotype. We confirmed the presence of these novel lncRNAs in chondrocytes and examined their expression in other joint tissues and during differentiation of hMSCs. Many are specifically induced during hMSC chondrogenesis. Of particular interest are a group of lncRNAs which surround the chondrocyte transcription factor SOX9 locus. Depletion of select lncRNAs by RNAi disrupted hMSC chondrogenesis, concomitant with reduced cartilage gene expression, including SOX9 itself, and incomplete matrix component production, indicating an essential role in chondrocyte biology.

Discussion We have characterised the chondrocyte non-coding transcriptome and identified novel lncRNAs. A number of robustly expressed chondrocyte lncRNAs have critical roles in chondrocyte development. Our ongoing examination into the mechanisms of lncRNA function will shed light on chondrocyte gene regulation.

Muscle aging and physiology in inclusion body myopathy

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Introduction Inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD; OMIM 167320) is an autosomal dominant inherited multisystem disorder caused by mutations in valosin-containing protein or VCP. The knock-in mice expressing the common human p.R155H VCP mutation develop a progressive myopathy with ubiquitin positive inclusion bodies, accumulation of abnormally appearing mitochondria in skeletal muscle and focal bone degradation reminiscent of Paget disease of the bone.

Materials and Methods To further assess the physiological effects of this mutation in muscle, we compared the *in vitro* contractile properties of the extensor digitorum longus (EDL) (fast-twitch muscle) and soleus (slow-twitch muscle) from mice heterozygous for the p.R155H mutation in VCP and wild-type mice.

Results Our results show that fast-twitch muscle fibres isolated from mutant mice ~12–15 months old not only fatigued faster and to a greater extent, but also recovered significantly slower and to a lesser degree than those of age-matched wild-type mice. Thereafter, the muscles seem to recover and by the time the mice were 27 months old, there was no difference in the fatigue resistance of mutant and wild type mice.
Discussion Our findings suggest that the effects of VCP mutation in skeletal muscle fibres may be fibre type-dependent and therefore that VCP may be necessary for maintenance of glycolytic capacity in mouse fast-twitch muscle fibres at 12–15 months only.

Re-equilibration of tenocyte tentional force is mediated by MMP-14

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Introduction In response to damage or rupture of the tendon tissue, the tensional forces between the cell and the extracellular matrix are reduced. Tenocytes are able to re-equilibrate this tension. The aim of the current study is to understand the mechanism that controls this re-equilibration.
Materials and Methods Tenocytes derived from human Achilles tendons using explants outgrowth were seeded at 0.5×10^6 cells/ml into collagen (rat tail type I, 1 mg/ml) gels, tenocyte seeded gels were released and contraction was measured over a 48 h period (contraction quantified by image J). Cytochalasin D, cyclohexamide, emetine, anisomysin and siRNA to specific MMPs were added before release (siRNA treatment 48 h before seeding into collagen). RNA and protein analysis was performed using quantitative real time PCR, Western blot and gelatine zymography.
Results Cytochalasin D completely abrogated tenocyte mediated contraction of collagen ($n = 3$, $P = 0.0006$). Protein synthesis inhibitors cyclohexamide, emitine and anisomycin also inhibited the contraction ($n = 3$, $P = 0.018$). MMP1, MMP2 and MMP14 were reduced at the protein and mRNA level in response to the protein synthesis inhibitors ($n = 3$, $P \leq 0.05$). Tenocytes were treated with MMP1, MMP2 and MMP14 siRNA. MMP1 and MMP2 siRNA had no significant effect upon contraction. MMP14 siRNA (>90% Knockdown) decreased the level of contraction by 15% ($n = 3$, $P = 0.023$).
Discussion The inhibition of contraction by cytochalsin D, an inhibitor of the actin cytoskeleton, indicates that contraction or re-equilibration of tensional force is regulated by the actin cytoskeleton. Inhibition of protein synthesis also inhibited contraction therefore newly synthesised protein is involved in the re-equilibration of tension. MMP1, MMP2 and MMP14 protein and mRNA levels were abrogated in the presence of protein synthesis inhibitors. This indicates that all three of these MMPs were newly synthesised. MMP14 siRNA reduced the level of contraction by 15%. Therefore, MMP14 is newly synthesised by tenocytes in response to stress relaxation and MMP14, as well as the actin cytoskeleton, plays a role in tenocyte mediated re-equilibration of the tensional forces between the cell and the matrix. This information may be important in understanding tenocyte mediated remodelling of the ECM after injury.

Cell migration in a chondroprogenitor cell line

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Introduction Chondroprogenitor cells naturally reside in cartilage and are able to migrate to sites of cartilage injury. The study of chondroprogenitor cells is therefore of interest to investigate not only the molecular signalling events that occur during chondrogenesis, but also the response of cartilage to damage and how cartilage may attempt to repair itself. ATDC5 chondroprogenitor cells have previously been shown to migrate in response to several growth factors in chemotaxis assays. Here the migratory potential of ATDC5 cells on different extracellular matrix substrates was assessed after culture in monolayer or in three-dimensional micromass. Differentiation was monitored at both mRNA and protein levels.
Materials and Methods ATDC5 cells were grown in either standard monolayer culture or in three-dimensional, high-density micromass, stimulated with insulin (which induces differentiation in ATDC5 cells) and ascorbic acid for 2 weeks. Migration of both undifferentiated and differentiated ATDC5 cells (dissociated from micromass cultures) was investigated on different substrates by time-lapse microscopy over 17 h. Quantitative (q)RT-PCR, western blotting and immunofluorescence were used to identify gene/protein regulation related to chondrogenesis and cartilage phenotype.
Results Undifferentiated cells migrated over a fibronectin-containing matrix but their migration was impeded by migration on Type II Collagen. Differentiated ATDC5 cells were less migratory than undifferentiated cells. Differentiation of ATDC5 cells was enhanced by 3D culture and ascorbic acid stimulation compared to standard culture in monolayer without ascorbic acid as indicated by the increase in typical markers of differentiation (including type II collagen) at both RNA and protein levels.
Discussion We showed that ATDC5 cells have a migratory phenotype, reminiscent of primary chondroprogenitor cells and that following micromass culture, ATDC5 cells show reduced migration whilst maintaining a differentiated phenotype. We therefore suggest ATDC5 cells provide a good model system for studying the migratory phenotype of chondroprogenitor cells and differentiated chondrocytes.

The role of Engrailed for epaxial–hypaxial muscle development and innervation, and for the evolution of vertebrate three-dimensional mobility

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Introduction Modern, jawed vertebrates (gnathostomes) evolved from primitive jawless vertebrates, and their basic movement pattern is swimming by side-to-side undulations, powered by muscle blocks on either side of the body. Yet gnathostomes evolved to become one of the most successful animal groups on the planet as they conquered not only marine and fresh waters, but also the land and the air. A corner stone in this success was the acquisition of full, three-dimensional mobility. This was facilitated by the establishment of distinct and separately innervated dorso-ventral (epaxial-hypaxial) muscle, which allowed refined side-to-side as well as upwards-downwards movements for the first time. In contrast, muscle in jawless vertebrates remained dorsoventrally continuous and hence, upwards-downwards movements are restricted and movement patterns are simpler. How the more complex gnathostome system is established during embryogenesis and how it may have evolved in the ancestors of modern vertebrates, is not known. However, in earlier studies we found that the expression of the Engrailed homeobox gene is associated with the formation of the epaxial-hypaxial compartment boundary. We now asked whether Engrailed has a function in the control of epaxial-hypaxial subdivision and innervation of muscle.

Materials and Methods We comparatively analysed Engrailed gene expression and innervation pattern in gnathostomes as divergent as mouse, chicken and zebrafish. Moreover, we performed gain-and loss-of function experiments for Engrailed in these animals, and analysed the resulting changes in muscle pattern and innervation.

Results Our data show that in all animals analysed, the expression domains of Engrailed is associated with the establishment of the epaxial-hypaxial boundary of muscle. Moreover, the outgrowing epaxial and hypaxial nerves orientate themselves with respect to this Engrailed expression domain. Loss and gain of function experiments in the chicken revealed that Engrailed controls the epaxial-hypaxial patterning of muscle. Importantly, in chicken, mouse and zebrafish, loss and gain of Engrailed function severely disrupted axonal pathfinding.

Discussion Our data suggest that the recruitment of Engrailed into the gnathostome somite facilitated the segregation and separate innervation of epaxial-hypaxial muscle and thereby the evolution of full three-dimensional mobility.

Sulfurophane represses matrix-degrading proteases and protects cartilage from destruction in vitro and in vivo

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Introduction Nutrition impacts directly on healthy ageing and obesity, both of which are major risk factors for osteoarthritis. Sulforaphane (SFN) is an isothiocyanate found in brassicas and has been reported to regulate signalling pathways relevant to chronic diseases. Our study investigated whether sulforaphane can abrogate cartilage destruction in laboratory models of osteoarthritis and examined mechanism of action in chondrocytes.

Materials and Methods The impact of SFN treatment on gene expression, signalling through transcription factors nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor kappaB (NFκB), and histone acetylation were examined in chondrocytes. The intracellular concentrations of SFN and SFN metabolites were quantified in chondrocytes and used to inform transcription factor binding assays. The bovine nasal cartilage explant model (BNC) and destabilisation of medial meniscus (DMM) murine model of osteoarthritis were used to study chondroprotection by SFN.

Results SFN inhibited cytokine-induced metalloproteinase expression in primary human articular chondrocytes (HACs) and in fibroblast-like synovial cells (FLS). SFN can act independently of the Nrf2 transcription factor and histone deacetylase activity in HACs, but does mediate prolonged activation of Jun kinase (JNK) and p38 MAP kinase. SFN attenuates NF-κB signalling through at least inhibition of DNA binding in HACs with attenuation of expression of several NF-κB dependent genes. SFN abrogates cytokine-induced destruction of bovine nasal cartilage at the level of both proteoglycan and collagen breakdown (10 μM compared to cytokines alone). It also decreases arthritis score in the DMM murine model of osteoarthritis (3 μmol daily dose SFN in diet versus control chow).

Discussion SFN, at levels which can be obtained through a high broccoli diet, inhibits the expression of key metalloproteinases implicated in osteoarthritis independently of Nrf2 and blocks inflammation at the level of NF-κB to protect against cartilage destruction *in vitro* and *in vivo*. Ongoing studies in man will ascertain the potential of this compound in human osteoarthritis.

Mechanosensitivity of embryonic limbs: mechano-sensitive and insensitive periods of growth, differential sensitivity between limb elements, and the underpinning mechanisms

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Introduction The role of mechanical input in maintaining adult skeletal structure and health is well established, but mechanical stimuli arising from embryonic muscle contraction is also critical for skeletal growth during development. We aimed to investigate the effect of in ovo immobilisation of embryonic chickens on the longitudinal growth of limb skeletal elements throughout development, in order to identify mechano-sensitive time-points, differential sensitivity between limb elements, and the mechanisms underpinning any alterations in growth observed.
Materials and Methods Embryonic chickens were immobilised at different stages of development between E10 and E18 using decamethonium bromide. Both immobilised and control embryos (treated with vehicle only) were monitored by daily MRI scans and measurements of limb element growth were plotted. Immunolabelling for proliferating cell nuclear antigen, TUNEL assay and imaging of hypertrophic zone chondrocytes with confocal microscopy in order to obtain cell volume measurements were used to evaluate any differences in proliferation, apoptosis and hypertrophy between control and immobilised limbs.
Results Mechanosensitivity appears to be acquired relatively late in development, around E14, and different limb elements show varying sensitivity to mechanical input. The greatest reduction in growth was observed in the most distal limb element at all time-points, and the middle elements of each limb i.e. tibia, radius and ulna appear relatively protected from immobilisation effects. The distribution of proliferating and apoptotic cells in limb element growth cartilage was altered by immobilisation, and chondrocyte volume in the hypertrophic zone of growth cartilage was reduced.
Discussion Mechanical input is involved in the regulation of endochondral ossification processes in normal development. Early, intrinsically regulated growth appears to switch to mechanically regulated growth at approx. E14. Our results suggest a greater sensitivity of distal limb elements to mechanical input. It is possible that plasticity in distal limb form in late stages of development is evolutionarily advantageous.

Collective cell behaviour in ECM stiffness sensing

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Introduction The stiffness of the extracellular matrix (ECM) has a profound effect on the behaviour of many cell types. Cells exert contractile force on ECMs and sense the resultant deformation that develops in the ECM. This is dependent not only on elastic modulus, but also on ECM thickness and heterogeneity, which means cells can mechanosense nearby features without direct contact. Here, we hypothesised that cohesive groups of cells would exert more force and be able to mechanosense features at greater distances than individual cells.
Materials and Methods We fabricated polyacrylamide gels of 50 or 1000 μm thickness and 0.5–40 kPa stiffness (measured by AFM) adhered to glass substrates using custom designed gel moulds. Gel surfaces were then covalently modified with ECM proteins (collagen and fibronectin). We plated MG63 osteoblasts or MDCK epithelial cells on gel substrates either at low density ($1000/cm^2$) or in compact colonies of ~4 mm diameter ($1.5 \times 10^5/cm^2$). We measured colony diameter, cell aspect, and cell perimeter by light microscopy and cell division by EdU staining.
Results The perimeter of MG63 and MDCK cells plated as individual cells at low density on 50 μm-thick gels increased as a function of substrate elastic modulus [from ~70 μm (2 kPa) to ~160 μm (40 kPa) for MG63 and from ~60 μm (0.5 kPa) to ~140 μm (40 kPa) for MDCK]. In contrast to this, no differences in the phenotype of cells within colonies was observed regardless of measured gel elastic modulus. When gel thickness was increased to 1000 μm, however, cells in colonies showed morphological differences dependent on gel elastic modulus. Cells were significantly denser at peripheral colony regions on thick, soft (0.5–2 kPa) gels than on stiff (40 kPa) gels, and had higher aspect ratios and perimeters.
Discussion These results support the notion that collective groups of cells are able to mechanosense rigid materials beneath elastic substrates at greater depths than individual cells. This raises the possibility that the collective action of cells in tissues such as epithelia may allow contributing cells to sense structures of differing stiffness at comparatively large distances. This phenomenon may act to direct cell patterning and differentiation during development or during tissue healing.

SirT1 promotes autophagy in chondrocytes and osteoblasts

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Introduction Advanced age is a powerful risk factor in musculoskeletal (MSK) disease, yet the role of ageing-related mechanisms in disorders such as osteoarthritis and osteoporosis remain poorly understood. Autophagy is a cellular process promoting "good housekeeping" which clears and recycles intra-cellular debris, and is decreased in ageing tissues and age-related disorders (including osteoarthritis and

osteoporosis). The class III histone deacetylase, SirT1 regulates cellular process linked to ageing and contributes to the lifespan extension properties of a calorie-restricted (CR) diet, an approach also reported to activate autophagy. Here we investigate the role of SirT1 in regulating autophagy in chondrocytes and osteoblasts.

Materials and Methods Gene (qPCR) and protein (Western blot) expression profiles of key autophagy markers (Beclin-1, ULK-1, LC3) were examined in chondrocyte (HTB-94) or osteoblast (2T3) cell lines after either pharmacological inhibition of SirT1 using EX-527 (100 nM), SirT1 siRNA knockdown or by pharmacological activation of SirT1 using SRT1720 (500 nM). Effects of SirT1 deletion and CR on the MSK system *in vivo* were assessed by microCT analysis and autophagy-related marker (LC3, by IHC).

Results Pharmacological inhibition and siRNA knockdown of SirT1 in chondrocyte HTB-94 cells decreased the gene expression levels of Beclin-1 ($P < 0.01$), ULK-1 ($P < 0.01$), LC3 ($P < 0.01$). In contrast, activating SirT1 in chondrocytes increased the gene expression of autophagy markers ($P < 0.01$). Furthermore, inhibiting SirT1 activity in osteoblasts decreased mineralised nodule formation and LC3 protein expression. Mice fed a CR diet had elevated SirT1 gene expression ($P < 0.001$), significantly higher trabecular bone volume (BV/TV 10.8% $\pm$ 0.4% compared to 6.7% $\pm$ 0.5% in *ad libitum* fed controls, $P < 0.0001$). Importantly, SirT1 deficient mice showed decreased expression of LC3 and failed to demonstrate any retention of trabecular bone following CR.

Discussion This data suggests that SirT1 regulates autophagy in chondrocyte and osteoblast cell lines and may mediate dietary effects, such as following CR, in the bone and joint by controlling autophagy in these cells.

A systems biology approach to understand the pathogenic role of the extracellular matrix in muscular dystrophy

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Introduction Skeletal muscle has a remarkable regenerative capacity; however, this can become exhausted upon repeated injuries or in the presence of chronic injury (e.g. in muscular dystrophy). Loss of muscle regenerative capacity is accompanied by increased extracellular matrix deposition, reduced blood supply to the tissue and often inflammation. Although several molecules that could possibly play a role in promoting extracellular matrix accumulation have been identified, especially in the context of muscular dystrophy, little is known about the structural changes occurring in the extracellular matrix of repeatedly injured and dystrophic muscles. Specifically, it is not known how changes in extracellular matrix composition, abundance and structure might affect muscle progenitor cell proliferation, migration and differentiation by capturing differential amounts of growth factors and cytokines.

Materials and Methods We used a systems biology approach (discovery proteomics followed by network analysis) and a mouse model of Duchenne muscular dystrophy (the mdx4cv mouse) to identify extracellular "soluble" factors that accumulate within the extracellular matrix as muscular dystrophy progresses.

Results We identified several extracellular proteins and defined candidate signalling pathways that were previously unexplored in the context of muscular dystrophy. We explored the role that these signalling pathways might play in muscular dystrophy pathogenesis, with a focus on satellite cell and fibroblast proliferation and differentiation.

Discussion Our results support a mechanistic model for muscular dystrophy pathogenesis whereby loss of muscle regenerative capacity is directly caused by altered extracellular matrix composition and structure, which in turn affects satellite cell and fibroblast homeostasis ultimately leading to impaired muscle repair.

The role of muscle activity on zebrafish jaw joint shape and cell behaviour

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Introduction The importance of muscle activity during the developmental process of joint morphogenesis has been shown in many studies. A lack of muscle activity leads to abnormal joint shaping and fusions. However, less is known about how this mechanical stimulus affects the underlying cell behaviour that ultimately causes the altered joint shape. We wanted to investigate the role of muscle on cell behaviour proximal to the joint. Clinically, abnormal joint morphogenesis is linked to conditions such as Developmental Dysplasia of the Hip (DDH) and osteoarthritis.

Materials and Methods Finite Element Analysis models were generated using Abaqus Software at 78–124 hpf to model the Maximum Principal Strain acting on the zebrafish jaw. Fish were anaesthetised at different stages between 78–128 hpf with MS222. ImageJ and PAST software were used to produce circular histograms to plot the orientation of cells at the joint.

Results Using zebrafish as a model, we show that removal of muscle activity through anaesthetisation or genetic manipulation causes a change in jaw joint shape. By sequentially removing and adding zebrafish from anaesthesia, we investigate the developmental periods that are critical for normal joint morphology. We have developed Finite Element Analysis models (FEA), to visualise the location of strains during joint morphogenesis. By investigating the effect of anaesthetisation on chondrocyte orientation at the jaw joint, coupled

with the strain map models, we can investigate the relationship between muscle activity and joint cell behaviour.

Anaesthetising fish at different stages of joint development revealed the timepoints that critically required muscle activity for joint shaping. Finite Element Analysis revealed that Maximum Principal Strain acted on the medial side of the jaw joint. Studying cell orientations in the joint, we observed differences in orientation only in those cells in positions corresponding to the highest strains.

Discussion There are critical time-points during joint morphogenesis where muscle activity is required to produce a normal joint shape. A switch in cell orientation at the medial joint correlates with the area that is usually under Maximum Principal Strain and which strongly suggests that muscle activity controls the orientation of cells in the joint.

Wnt-signalling in human articular chondrocytes

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Introduction Aberrant Wnt-signalling in cartilage results in chondrocyte dedifferentiation, hypertrophy, loss of type II collagen, aggrecan and glycosaminoglycans. Articular chondrocytes experience a pO2 gradient from ~6% (surface) to ~1% (within deeper layers). Hypoxia is known to modulate Wnt-signalling. This study investigates how the Wnt profile alters in human articular chondrocytes (HACs) in response to Lithium Chloride (LiCl), a stimulator of canonical Wnt-signalling, during normoxia and hypoxia.

Materials and Methods HACs were isolated from macroscopically normal femoral condyle cartilage at the time of joint replacement. HACs were cultures and passaged prior to LiCl exposure at 0, 20, 40 or 80 mM for 3, 8 and 24 h in normoxic or hypoxic conditions (1%, 24 h only). Culture medium was assayed for secreted DKK-1 by ELISA and RNA was extracted. Real-time qPCR was performed to analyse the expression of DKK-1, Wnt3a, Wnt5a, Wnt7b, Wnt10, Wnt11 and Wnt16, using GAPDH as a reference gene. A threshold of two-fold change in expression (either up- or down-regulated) was deemed of biological significance. Localisation of ß-Catenin was analysed by immunocytochemistry using a specific monoclonal antibody against the active (unphosphorylated) form of ß-Catenin.

Results Increasing concentrations of LiCl resulted in down-regulation of DKK-1 and up-regulation of Wnt3a, Wnt5a, Wnt10 and Wnt11. Expression levels reached and maintained the expression threshold or above by 3hrs for DKK-1 and Wnt3a, 8 h for Wnt10 and Wnt11 and 24 h for Wnt5a. There was a significant increase in Wnt11 expression at 40 mM LiCl under hypoxia versus normoxia. No other effect of hypoxia was observed. Wnt7b and Wnt16 were not detected. Secreted DKK-1 and nuclear translocation of ß-Catenin were decreased and increased respectively in a time- and dose-dependent manner.

Discussion LiCl induces canonical Wnt-signalling by increasing nuclear translocation of ß-Catenin and reducing its inhibitor, DKK-1. LiCl also induced the up-regulation of both canonical (Wnt3a and Wnt10) and non-canonical (Wnt5a and Wnt11) signalling ligands. Hypoxia affected the expression of Wnt11 only when compared to normoxia. *In vitro* modulation of Wnt-signalling lends the opportunity to investigate the effects of increased Wnt-signalling on chondrocyte behaviour and how this may be manipulated for the treatment or prevention of osteoarthritis.

The expression of toll-like receptors on mesenchymal stem cells isolated from human umbilical cord

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Introduction Toll-like receptors (TLRs), primarily expressed on antigen presenting cells, lead to the production of anti-inflammatory and pro-inflammatory proteins upon ligand stimulation. Expression of TLR3 and 4 on MSCs is associated with polarisation towards anti-inflammatory or pro-inflammatory phenotypes respectively1. TLR3 is associated with viral RNA and TLR4 is associated with bacterial lipopolysaccharide (LPS). This study investigated TLR3/4 expression and the immunoregulatory activity on umbilical cord (UC-MSCs) and bone marrow MSCs (BMSCs) primed with or without TLR ligands and the pro-inflammatory cytokine IFN-γ.

Materials and Methods UC-MSCs (n = 5, from whole UC) and BMSCs (n = 2, from iliac crest). Upon reaching 70–80% confluence, cells were treated for 24 h with the following; LPS (10 ng/ml) ± IFN-γ (25 ng/ml), polyinosinic:polycytidylic acid ([Poly(I:C)], 1 μg/ml) ± IFN-γ (25 ng/ml) or IFN-γ (25 ng/ml), using untreated cells in normal medium as control. After 24 h, RNA was extracted and real-time qPCR performed to either analyse TLR3/4 expression using GAPDH as a reference gene (n = 7) or a TLR-signalling PCR-array (n = 1, UC-MSC normal media ± IFN-γ). A threshold of two-fold change (up/down-regulated) was deemed biologically significant.

Results TLR3 was up-regulated in response to IFN-γ ± Poly (I:C) but with Poly(I:C) alone, expression was variable across all samples. TLR4 expression (in response to IFN-γ+LPS) was also variable. One BMSC sample up-regulated TLR4 in response to IFN-γ alone and one UC-MSC down-regulated TLR4 in response to IFN-γ+LPS. The PCR-array showed a down-regulation of 29 genes in UC-MSC in response to IFN-γ, including TLR1/2/4/5/6/7/8/9/10, CD80 and 86 and IL8, IFN-γ and TNF. Eight genes were up-regulated including TLR3, IRF1, IL6, CXCL10, CCL2 and IL12A. HLA-G expression was not inhibited by either Poly (I:C) or LPS.

Discussion These results demonstrate both cell type and patient variability of TLR3/4 expression. IFN-γ up-regulated TLR3 expression as expected, whilst the effect of Poly(I:C) was variable. LPS did not up-regulate TLR4 in UC-MSCs. IFN-γ down-regulated pro-inflammatory cytokines (such as IL8 and TNF) and up-regulated other inflammatory cytokines (such as IL6 and IL12A). These preliminary results indicate that TLR3/4 expression could lead to a more pro-inflammatory phenotype, despite HLA-G expression remaining unchanged regardless of stimuli.

Methods for the identification of MicroRNA-455 targets

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Introduction MicroRNAs (miRNAs) have emerged as a new class of gene expression regulators that are important in both normal cartilage physiology and pathology. We have previously identified several miRNAs that are regulated in osteoarthritis, including miRNA-455, a key Sox9-responsive miRNA. MiRNAs are 20–24 nucleotide non-coding RNA molecules that post-transcriptionally regulate gene expression. MiRNAs are known simultaneously to target many transcripts, regulating complex signalling pathways. A single miRNA can regulate multiple mRNAs via binding to sequences in the 3′UTR either promoting mRNA decay or repressing translation. mRNA profiling can be used to identify some miRNA targets, but fails to identify targets that change only at the protein level and to distinguish direct from indirect targets. Using whole genome array analysis we have identified genes that are regulated at the mRNA level or the protein level.
Materials and Methods Primary human articular chondrocytes were transfected with either 50 nM miR-455-3p mimic or inhibitor or non-targeting controls. Cells were incubated for 48 h and harvested for mRNA using Trizol (Invitrogen). Illumina whole genome micro-array analysis was performed to identify genes regulated at the mRNA level. We further analysed the 3′ UTRs for mR-455 binding sites. To identify genes that only change at the protein level we analysed the promoter sequences of regulated genes for common binding sites for transcription factors.
Results Genes where the mRNA was both decreased by over-expression of miR-455-3p and increased by its inhibition, and contain a miR-455-3p-binding site, include members of the WNT signalling pathway including DKK1, beta-catenin, and GSK. Following analysis of the promoter sequences of regulated mRNA, two motifs were identified, CACGTG and CAGGTG which are binding sites for the transcription factor TCF3, itself implicated in WNT signalling. Luciferase assays confirmed these genes as direct targets of miR-455-3p.
Discussion We have combined different bioinformatic analyses of mRNA datasets to identify direct targets of miR-455-3p which change at the mRNA level or only at the protein level. Pathway analysis has revealed that miR-455 may regulate multiple points in the WNT signalling pathway.

Investigation into cell specific responses during cellular interactions between equine fibroblast-like synoviocytes and canine macrophages in an inflammatory environment – development of a synovial co-culture model system

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Introduction Synovitis is a key mediator of osteoarthritis, and in the perpetuation of cartilage degradation. Synovial inflammation is characterised by increases in catabolic cytokines, notably TNF-α and IL-1β, which are predominantly produced by synovial macrophages. These cytokines cause an increase in fibroblast-like synoviocyte (FLS) gene expression of IL6, IL8, MMP1 and MMP3. ADAMTS4 gene expression is also influenced by IL-1β, whilst ADAMTS5 is thought to be unaffected, and constitutively expressed by chondrocytes.
Materials and Methods To investigate inter-cellular signalling in the inflammatory response, three conditions were examined. (1) An optimised co-culture model using equine FLS (EFLS) and canine macrophages (DH82 cells). Gene knockdown in DH82 cells prior to co-culture was also investigated. (2) EFLS exposed to conditioned medium from DH82 cells. (3) EFLS and DH82 co-cultured without contact using well-inserts. All conditions were exposed to 10 μg/ml lipopolysaccharide (LPS) for 12–24 h. Cell specific responses were determined via qRT-PCR using species-specific primers.
Results LPS stimulation of EFLS caused rapid increases in IL-1β, IL6, ADAMTS4 and ADAMTS5 mRNAs. Stimulation was similar when co-cultured with DH82 cells, except for ADAMTS5, which was significantly diminished. When EFLS were cultured with DH82 cells in transwells, or with DH82 conditioned media; the ADAMTS5 response to LPS stimulation was similarly reduced. Knockdown of IL-1β in DH82 cells did not change this inhibitory effect.
Discussion We conclude that macrophages can influence FLS gene expression through a soluble mediator; likely to be IL-1β independent, and that inter-cellular signalling suppresses FLS ADAMTS5 gene expression. This supports previous reports suggesting that IL-1β, predominantly produced by macrophages, does not influence ADAMTS5 expression, unlike ADAMTS4, which is upregulated[2]. There was significant and effective knockdown of IL-1β in DH82 cells, which further supported this conclusion as EFLS ADAMTS5 gene expression was unaffected. Work is now underway to determine the signalling pathways that mediate this suppression.

Investigating the role of Wnt antagonist protein Dkk-1 in the pathogenesis of osteoarthritis

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Introduction Osteoarthritis (OA) is a degenerative disease affecting synovial joint leading to articular cartilage destruction and bone remodeling. It is the most common form of polyarthritis in the UK. Current management of end-stage OA involves joint arthroplasty that have several limitations. Therefore, there is a shift for cell-based therapies in the form of stem/progenitor cells for cartilage regeneration and to understand regulatory pathways involved in pathogenesis of OA.
Materials and Methods: Human knee cartilages, healthy and from patients with OA were obtained from donors with institutional ethical approval and chondrocytes were isolated using sequential pronase/collagenase digestion. Isolated chondrocytes were subjected to fibronectin adhesion assay to isolate cartilage progenitor clonal cell (CPCs) populations. Bone marrow-mesenchymal stem cells (BM-MSC) were isolated using density gradient centrifugation. Osteochondral tissue was used for immunohistochemical staining of Dkk-1.
Results CPCs and BM-MSCs were successfully isolated from cartilage and/or bone marrow taken from normal and osteoarthritic donors. Initial comparison of both cell types showed similar morphology and proliferation capacity. In addition both cell types isolated showed positive expression for the putative stem cell makers; CD-90, CD-105 and CD-166 while lacking expression of CD-34. Furthermore, by studying RNA isolated from normal and osteoarthritic CPCs, our preliminary data indicates that the mRNA expression levels for Dkk1, Wnt antagonist protein, is increased in CPCs isolated from osteoarthritic subjects relative to chondrocyte progenitor cells isolated from normal cartilage. This finding was further supported by an increased in secreted Dkk-1 protein from OA CPCs and immunohistochemical staining of Dkk-1 protein showing an increased in immunohistochemical staining in osteoarthritic cartilage. Moreover, immunolocalisation of Dkk-1 in osteochondral tissues have shown that in addition to cartilage cluster cells expressing Dkk-1, there was also expression of Dkk-1 around migratory blood vessels from the underlying subchondral bone. *In vitro* angiogenesis assay confirmed that Dkk1 has promoted the angiogenic and migratory role of endothelial cells. By studying the chondrogenic potential of isolated CPCs, CPCs with high levels of Dkk-1 failed to undergo chondrogenic differentiation.
Discussion Our results indicate that the canonical Wnt antagonist Dkk-1 is up-regulated in OA CPCs and that it might play an important role in the pathogenesis of OA by promoting angiogenesis.

The mechanism of, and therapeutic strategies to treat, collagen IV kidney disease

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Introduction Collagen IV is a major component of basement membranes (BM) and Col4a1 mutations cause vascular, eye and kidney disease including HANAC syndrome (hereditary angiopathy with nephropathy, aneurysm and cramps syndrome). However the disease mechanisms of COL4A1 mutations and their role in kidney disease remain poorly understood.
Materials and Methods All experiments were performed on female mice with a missense mutation in Col4a1. Defects in renal structure and function were assessed via histopathology and *in vivo* renal function, respectively. Metabolic cage study, dispstick analysis and ELISA were used for urine analysis. Protein levels were measured via Western blotting and electron microscopy was used to determine BM structure. BM composition was analysed by immunohistochemisty. Chemical chaperones were administered orally for 1 month on 3 month old mice.
Results Previous initial analysis of renal disease in Col4a1 mutant mice revealed defects in the glomerulus and proteinuria. Here, we show that the glomerulopathy is age dependent and accompanied by atrophy of the medulla which leads to polyuria (increased urine production). The polyuria is caused by a urine concentrating defect which results from an atrophy driven reduction in the expression of the aquaporin 2 channel. The increased water excretion occurs in the presence of increased sodium reabsorption in the proximal tubules via the sodium hydrogen exchanger (NHE3), indicating a breakdown in water-electrolyte balance. Further analysis revealed that these phenotypes were associated with ER-stress, due to the intracellular accumulation of mutant collagen, and BM defects. Intriguingly BM defects were absent in the proximal tubules, implying that ER-stress may play a causative role. To investigate this hypothesis, mice were treated with a chemical chaperone that we previously showed to reduce ER-stress in collagen IV mutant cells. Critically, treatment decreased ER-stress and haematuria, suggesting that it has efficacy in alleviating at least some of the renal phenotypes caused by collagen IV mutations.
Discussion These results highlight additional kidney phenotypes which may develop in patients and provide the first mechanistic insights into Col4a1 kidney disease. Furthermore, they suggest targeting intracellular accumulation of mutant collagen IV as a future therapeutic strategy.

Identification and characterisation of novel MicroRNAs in osteoarthritis

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Introduction Osteoarthritis is a painful and debilitating disease characterised by loss of cartilage, aberrant gene expression and bone remodelling. Many of the signalling pathways and transcription factors which control chondrocyte development as well as the joint disease have been established. MicroRNAs are very small, 20–24 nucleotide non-coding RNA molecules that post-transcriptionally regulate gene expression. There is evidence to suggest that they can affect chondrogenesis and both the initiation and progression of osteoarthritis, however the exact mechanisms are still mostly undetermined. At the moment it is unclear exactly how many may be expressed in cartilage.
Materials and Methods A small RNA library was prepared from osteoarthritic primary chondrocytes using in-house "high definition" adaptors and analysed by Illumina next generation sequencing. Novel candidate microRNAs were validated by northern blot and qRT-PCR. Expression was measured in human cartilage, across chondrocyte de-differentiation, in chondrogenesis and after cartilage injury. Predicted targets of novel candidates were identified by microarray and computational analysis, and validated using 3′-UTR Luciferase reporter plasmids containing either wild-type or mutant miRNA target sequence.
Results We identified 990 previously identified annotated microRNAs and 1621 potential novel microRNA candidates in osteoarthritic chondrocytes, with 61 expressed in all three samples assayed. MicroRNA-140-3p showed the highest expression value. Sixteen novel candidate microRNAs were analysed further using validation techniques including qRT-PCR and northern blotting, and three were determined to be true microRNAs. Target analysis combining microarray and computational analysis resulted in hundreds of potential targets, with a number validated by qRT-PCR, and ITGA5 being experimentally validated by Luciferase reporter assays as a genuine target of candidate microRNA novel 11.
Discussion Deep sequencing has uncovered many potential microRNA candidates expressed in human cartilage and has provided an extensive analysis and increased understanding of the miRNome of osteoarthritis. For at least three of the newly discovered microRNAs, the pattern of expression makes them of potential functional interest in cartilage homeostasis and osteoarthritis.

Lovastatin–mediated changes in human tendon cells

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Introduction Statins are a cholesterol-lowering drug family prescribed to reduce the risk of cardiovascular disease. They are well tolerated. Side effects are rare and mainly muscle related. Although cholesterol reduction is thought to be the primary mechanism underlying the benefits of statin therapy, statins have been shown to act also through a lipid-independent mechanism. By inhibiting cholesterol production, statins interfere with isoprenoid synthesis. Although the mechanism for muscle-specific adverse effects is not well understood, cholesterol-independent action of statins may play role. Recently some potential adverse effects were reported also in tendon. The aim of the study was to explore tendon-specific effects of statins *in vitro* using human tenocytes.
Materials and Methods The viability of human tenocytes in presence of 0.2–2 μM lovastatin was measured using alamarBlue and live/dead cell assay. Changes in expression of COL1A1, COL3A1 and BMP-2 in lovastatin-treated cells were determined by qPCR. Scratch assay was employed to evaluate the migration of tenocytes in presence of the drug. To measure intercellular communication the cells were loaded with a calcein solution and subjected to FRAP using Zeiss LSM710 Confocal Microscope. Western blotting was used to confirm the effect of lovastatin on isoprenylation.
Results No changes in cell viability and shape were observed in human tenocytes treated with a therapeutic dose of lovastatin. Short-term exposure to lovastatin concentrations outside the therapeutic range had no effect on tenocyte viability, however decreased migration and induced some cells to round up. In tenocytes retaining fibroblastic morphology a prolonged exposure to a supratherapeutic concentration of lovastatin did not stop intercellular communication, although a decrease in communication probably due to reduction cell density was noted. Furthermore, lovastatin-treated cells up-regulated expression of osteogenic factor BMP-2 and decreased mRNA levels of type I and III collagen. Finally, mevalonate, a downstream metabolite in the cholesterol biosynthesis pathway that statins inhibit, was able to reverse lovastatin-induced changes in the cells.
Discussion In this study we showed that lovastatin in a dose-dependent manner decreases migration of human tenocytes and alters their expression profile, but does not influence intercellular communication. Further studies are required to investigate the effects of chronic statin treatment in tenocytes.

BBSMB/BSDB joint meeting report

by Danielle Blackwell and Gi Fay Mok

The Musculoskeletal System: from development to disease

(1-3 September 2014, University of East Anglia, Norwich)

The first joint meeting of the British Societies for Matrix Biology (BSMB) and Developmental Biology (BSDB) was, in our opinion, a great success. The meeting was held over three days, which were packed with brilliant science from different areas of musculoskeletal research, presented in talks, posters and general discussions. The conference, attended by around 120 delegates, was held in the brand new Julian Study Centre at the University of East Anglia, in a comfortable and well-equipped lecture theatre, with separate rooms for posters and exhibitors and break out spaces for coffee, snacks and mingling.

The **opening Keynote** speaker was Tom Rando (Stanford) whose lecture was sponsored by the International Society for Developmental Biology and Mechanisms of Development. His presentation provided an awesome start to the conference with a fascinating account of his group's work into a primed state of quiescent stem cells in muscle (Satellite cells). This turned out to be one of the highlights of the conference for me (DB), as it is closely related to my own research.

The Keynote was followed by an exciting afternoon of presentations within the area of **'Signalling and Development'**. Standout talks from this session included Gabrielle Kardon (Utah) on the development of the diaphragm, which is impaired in congenital diaphragmatic hernias, a condition with high mortality rates where holes develop in the diaphragm. Her lab uses elegant mouse genetics approaches to dissect the contributions of connective tissues and muscle to this severe phenotype. Malcolm Logan (London) talked on the advanced imaging and mouse genetics approaches they use to examine the cellular events regulating muscle and tendon formation in the limb, and Christine Hartmann (Münster) presented a detailed analysis of the role of Wnt signalling during trabecular bone formation.

One great feature of the conference was the opportunity offered to attendees, including PhD students and post-docs, to present their work in **short talks** after selection from submitted abstracts. Therefore, I (GFM) was able to present my work on microRNA mediated regulation of chromatin regulators during muscle development. I was also awarded one of three poster prizes, sponsored by the International Journal of Experimental Pathology (IJEP), on this topic. Other speakers in this session were Anne-Gaelle Borycki (Sheffield), who spoke about the composition of the muscle stem cell niche. Her student, Daniel Ranaldi, also won a prize for his excellent poster on this work. Dylan Sweetman (Nottingham) presented on the signals that activate the skeletal muscle programme in limb muscles, Susanne Dietrich (Portsmouth) discussed the role of innervation of hypaxial and epaxial muscles for the evolution of 3D mobility, and Clare Thompson (London) and Sue Kimber (Manchester) presented on signals in chondrocytes and their differentiation from pluripotent stem cell for the purpose of cartilage repair.

The first day ended with a lively **evening poster session and reception**, where the atmosphere, fuelled by a couple of glasses of free wine and nibbles, was bustling with stimulating conversations. This was my (DB) first opportunity to present some of my PhD work in the form of a poster and the sessions allowed for brilliant discussions with other students working in the field, but also with experienced researchers. It felt great to be able to discuss and get feedback on my work from my peers.

The second day consisted of two sessions. The first focussed on **'Mechanobiology and Anatomy'** and started with a fascinating talk from Eli Zelzer (Israel), with his group's novel take on the mechanics of fracture repair. Other great talks included Paul O'Higgins (York), who presented approaches to use finite element analysis to simulate bone deformations under loading conditions, leading to the variation of form during evolution. Chrissy Hammond (Bristol) gave a really interesting talk on the importance of mechanical loading for jaw development in

zebrafish and her student, Lucy Brunt, won the third poster prize for her presentation of this work. Mario Giorgi (London) presented on the role of fetal movement in hip joint morphogenesis and his selected talk was awarded a prize for best oral presentation by a young scientist, which was sponsored by Orthopaedic Research UK. After a quick coffee break, we heard impressive talks by Ronen Schweitzer (Portland) on tendon growth in the mouse limb and Andy Pitsillides (London) on his group's work on mechanical stimuli that are important for limb growth and joint formation. Additional short talks in this session were presented by Angela Yiu (London) on Fat-Hippo signalling in bone development, Cornelia Stein (Cologne) on hemicentin ECM proteins for the development and function of the myotendinous junction in mouse and zebrafish, Herbert Tempfer (Salzburg) on the origin of tendon cells, and Rebecca Rolfe (Dublin) on the impact of muscle contractions for ossification and joint formation.

The afternoon session was on **'Human Genetics and Pathology'** with talks by Mike Briggs (Newcastle), Madeleine Durbeej (Lund), Linda Troeberg (Oxford) and an IJEP-sponsored lecture by Veronique Lefebvre (Cleveland). The presentations offered great insights into the current research into the molecular and genetic bases for muscular dystrophies and joint pathologies, as well as the molecular regulation of normal cartilage and joint development. Short talks completed this session and were given by Qing-Jun Meng (Manchester), Pradeep Kumar Sacitharan (Oxford) and Carole Proctor (Newcastle) on tissue specific circadian clocks, and the molecular drivers of joint inflammation and ageing cartilage.

Another real 'plus point' for the meeting was that it enabled interactions of developmental biologists with matrix biologists and human geneticists to share ideas with each other, thus initiating stimulating discussions and possible collaborations. This was in part facilitated by the evening's **conference dinner**, which was held at St. Andrews Hall, a grade 1 listed building dating back to the 14th Century in the centre of Norwich. The Hall provided a great venue and atmosphere for more relaxed discussions of the days events.

The final day played host to the final session on **'Transcriptional and Epigenetic Regulation'** with great talks by Cay Kielty (Manchester) on genotypes of fibrillinopathies and Simon Tew (Liverpool) on the importance of RNA stability and turnover for chondrocyte biology. Short talks were given by Matt Barter (Newcastle) on the role of a novel long non-coding RNA in cartilage and Linh Le (Norwich) on the microRNA-29 family in osteoarthritis. The BSMB also awarded their Society's Young Investigator Award to Blandine Poulet (London) and she gave a lovely talk on her work on modelling osteoarthritis. The **final Keynote**, sponsored by Developmental Dynamics, was delivered by David Glass (Novartis, Boston), who gave the most fascinating and entertaining talk on his group's work in developing an antibody treatment for patients with muscular atrophies. This was another highlight to end a fantastic conference, which covered the full breadth from developmental biology to pathology and therapeutic approaches in the musculoskeletal system.

We would like to give our thanks and congratulations to the organizers of this first joint meeting of the BSMB and BSDB, which we thought was really **well balanced and of interest for members of both societies**. We are grateful to the panel composed of invited speakers judging eligible posters and short talks, and thank the many commercial sponsors who made this meeting possible with their support. We look forward to future opportunities for joint meetings.

Finally, we would like to thank the **BSDB/Company of Biologists** for providing travel awards for us to attend.

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Awards in 2014

The BSDB would like to congratulate all awardees of 2014 including the Waddington Medal winner Phil Ingham (*see: bsdb.org/awards/the-waddington-medal*), the Beddington Medal winner William Razzell (*see: bsdb.org/awards/the-beddington-medal*), the 10 Gurdon Summer Studentship winners Ashley Bae (*host: Ildiko Somorjai, St Andrews*), George Hunt (*Ian Hope, Leeds*), Ariana Mihai (*Val Wilson, Edinburgh*), Benedetta Carbone (*Keisuke Kaji, Edinburgh*), Lydia Dugmore (*Iwan Evans, Sheffield*), Sian Martin (*Susanne Dietrich, Portsmouth*), Rachel Turner (*Alistair McGregor, Oxford*), Kadri Oras (*Martin Collinson, Aberdeen*), George Choa (*Claudio Stern, UCL*), Kwok Lun Man (*Arantza Barrios, UCL*), and the BSDB poster prize winners at the last joint Spring meeting: Ms. Z.M. Löf-Öhlin, Mr. J.M. Grice and Mr. M. Figueiredo-Larsen.