2nd Joint Meeting of the British Society for Matrix Biology and Bone Research Society: Sunday 14th - Tuesday 16th June, 2009

Our Autumn and Spring BSMB Meetings of 2009 were combined and held jointly, for only a second time, with the Bone Research Society at UCL London, in the heart of the UK capital on June 14th, 15th and 16th. The broad theme of this meeting was '**Bone and Cartilage Biology - lessons from development, vascular interactions and dysplasias'**.

The meeting was organised and hosted jointly by Andy Pitsillides and Tonia Vincent (BSMB), and Tim Arnett and Sanjeev Patel (BRS). It attracted almost 350 delegates - probably the largest ever BSMB meeting - and financial support was gratefully received from: Amgen, Novartis Pharmaceuticals and Proctor and Gamble Pharmaceuticals (Gold sponsors); the Company of Biologists, Eli Lilly, Roche and Servier (Bronze sponsors) as well as Advanced Molecular Vision, e2v Scientific Instruments/Skyscan, IDS, PeproTech, Promocell, Scanco and Wyeth. The 3-day long programme featured: parallel society sessions, a concomitant BRS clinical day, the novelty of shorter (and some very short) oral BSMB poster presentations and a meet-the-Prof in the pub session (gorgeous sunny evenings led to their relocation to UCL with cold beers provided). The programme included talks from many international and national leaders in bone, cartilage and matrix biology. Joint symposia included: New Imaging technologies, Life in an Extreme Environment and a New Investigator Trainee session (another new experience for us and by all accounts a great success) on the Sunday; New Frontiers, the Control of Angiogenesis and a scientifically-inspiring lecture by Prof Tim Hardingham, recipient of our 2009 Fell-Muir Award and a typically, dogma-challenging inaugural Dent Lecture by Prof Alan Boyde (BRS) on the Monday. This balance of cartilage and bone was continued on Tuesday with Joint Symposia on Skeletal Development and Dynamic ECM relationships: Lessons from Dysplasias, and a BSMB session on Matricellular Proteins. These plenary sessions were complimented by some 25 short talks (further 25 from BRS) giving much-increased scope for our younger members to present their work.

The team of organisors is also very grateful to Graham Riley for his tireless support and expert management of the fantastic on-line abstract submission and registration service and to Janet Crompton for her diligence, professionalism and experience throughout. We are also grateful for the contributions made by post-docs, students and staff from both UCL and the Royal Veterinary College for the day-to-day smooth running of the meeting. A great compliment is also due to the supportive BSMB committee - it is clear that BRS would be very happy to consider similar joint meetings in the future.

Report prepared by bursary award winners Karen Brakspear, Kirsty Culley and Rose Davidson, Nidhi Sofat and Simon Tew

Sunday:

Joint Symposium: New imaging technologies for cartilage and bone

The session was opened by **Dr Rob van't Hof** (University of Edinburgh, UK) with a presentation entitled 'Using µCT to study bone architecture'. Dr van't Hof discussed the limitations of current standard bone imaging methods such as X-ray and DEXA scanning, including their inability to provide adequate 3D bone architecture information or sufficient resolution for imaging of individual trabeculae. Dr van't Hof presented µCT imaging as an alternative technology that can reach a very high degree of resolution (<5µm) and allow visualisation of bone microarchitecture in even small mammals such as mice. In this way, natural contrast between water rich tissues, fat and air can also allow lungs and fat to be visualised. Micro-CT uses an X-ray source to create cross-sections of a rotating sample that are then reconstructed to generate a 3D model using a computer algorithm. Micro-CT can provide various output including % bone volume (BV/TV), trabecular thickness

(Tb.Th), trabecular number (Tb.N), trabecular spacing (Tb.Sp), trabecular pattern factor (Tb.Pf) and the structure model index (SMI). This imaging technique can also be applied *in vivo* using a rotating X-ray source and camera and a static animal. However, the resolution is slightly reduced leading to a loss of trabecular detail and despite anaesthesia, small movements of the animal can distort the analysis. Increased resolution is possible by decreasing the rotation step angle, however this results in a longer scan time, generates large file sizes and only limited RAM is available for the analysis. Dr van't Hof demonstrated how µCT was being used in his lab to image mouse models including nNOS knockout mice which display increased % bone volume, increased trabecular volume and decreased trabecular spacing. Furthermore, µCT imaging showed that oviariectomy-induced bone loss was accelerated in these animals compared to wild-type. In another study, lesions were detected by µCT in mice with mutations in sequestesome 1 (SQSTM1), a gene associated with Paget's disease which is characterised by excessive and uncoordinated bone remodelling. Dr van't Hof concluded his presentation by stressing that while µCT provides a great deal of information, classic histomorphology remains necessary since µCT cannot tell us why bones are different or whether differences observed are due to changes in bone formation or bone loss.

The second talk of the session was presented by **Professor Alan Boyde** (London, UK) and entitled 'Imaging of osteochondral tissues'. Professor Boyde demonstrated the powerful nature of Scanning Electron Microscopy (SEM) for imaging the structure of bone at very high resolution (sub-micron) with research showing bone loss in hamsters as a result of hibernation. The presentation also showed that it was possible to generate casts of bone in order to study bone spaces and connectivity. Using this method, the remodelling process can be observed microscopically and the locations of blood vessels within bone detected. Images of bone space casts have also been used to show intra-trabecular blood vessels in large animals and humans and the replacement of mineralised articular cartilage with bone. Professor Boyde also showed images of an osteoblast sheet from below, where it was possible to see the points of osteocyte process connections and osteoclasts resorbing bone at multiple places at once. Professor Boyde next introduced Dynamic Aperture Microscopy which can be used on bone sections to generate images with a continuous apparent tilting motion, allowing a partial 3D view. This approach has shown that osteoblasts are actually flat and elongated when making bone matrix and collagen. Professor Boyde showed images indicating that osteoclasts liberate live osteocytes when resorbing bone and that osteocytes threatened by resorption will defend the walls of their lacunae homes until the last moment. In order to assess the orientations of collagen fibrils in bone sections, the specimen can be viewed with polarised light. Collagen is anisotropic which means fibres aligned transverse to the section will not change the refraction of light exiting the specimen, resulting in maximum brightness. When fibres are oriented along the axis of light, no refraction occurs and the specimen appears dark. However, it has become apparent that one problem with this technique is that the pattern of light and dark is actually coming from the whole section thickness rather than just the plane of focus. To finish, Professor Boyde briefly mentioned that bone mineral content at the microscopic scale could be determined by backscattered electron imaging.

The symposium was concluded by **Professor Leif Dahlberg** (Malmö University Hospital, Lund University, Sweden). His presentation entitled 'Towards understanding of osteoarthritis and joint health by contrast-enhanced MRI of cartilage' introduced a new imaging technique that allows cartilage quality and mechanical properties to be estimated by measuring cartilage aggrecan content. Aggrecan consists of a core protein with negatively charged glycosaminoglycans (GAG) attached. The GAGs draw water into the tissue which generates an osmotic pressure that can be counteracted by the tensile properties of type II collagen fibres and this allows cartilage to dissipate load and resist shear and compressive stresses. In his talk, Professor Dahlberg showed that GAG content could be measured by delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC). Distribution of a negatively charged contrast medium (Gd-DTPA2-) is inversely related to GAG

content, with areas of low GAG content accumulating a greater concentration of Gd-DTPA2-. This method was used to assess the effects of moderate exercise on the cartilage of a group of subjects previously menisectomised and therefore at risk of developing osteoarthritis. GAG content was improved to a greater extent in individuals of the exercise group compared to the control group, and a strong correlation was observed between GAG content and self-reported changes in physical activity showing *in vivo* that cartilage is dynamic and can be modified by exercise. In another menisectomised cohort, dGEMRIC was used to relate cartilage quality to OA risk factors including weight and muscle function. Increased weight (BMI>25) correlated with poor GAG content and good thigh muscle strength was associated with higher cartilage GAG content demonstrating high BMI is a cartilage deteriorating factor, while strong thigh muscles may have a protective effect on the cartilage quality. In patients with MRI verified ACL injury, dGEMRIC showed decreased GAG content in the medial compartment, where most ACL patients are at risk of developing OA. Professor Dahlberg provided further evidence that dGEMRIC could be used as a clinically relevant measure of cartilage integrity and a measure of low GAG content could be predictive of knee OA development. 17 subjects suffering knee pain and arthroscopic cartilage changes were examined by dGEMRIC and reassessed 6 years later. In knees developing radiographic OA changes, the original dGEMRIC index had been lower than in subjects that did not develop radiographic OA changes.

Joint Symposium: Life in an extreme environment

The first presentation of this session was from **Dr Irving Shapiro** (Thomas Jefferson Univeristy, Philadelphia, USA) and entitled 'Earth (Water), Wind (Oxygen) and Fire (Energy): a recipe for cell survival in a hostile environment'. Chondrocytes in the growth plate, articular cartilage and nucleus pulposus (NP) of the intervetebral disc exist in microenvironments with limited oxygen (wind) and nutrient supplies (fire) and high osmotic pressure (water). Dr Shapiro put forward the hypothesis that cells in such environments survive by displaying a state of autophagy which can be regulated by cross-talk between HIF-1α, mTOR and AMPK. Autophagy promotes cell survival by catabolism of the cells own components, generating energy. Punctate expression of the autophagic proteins Beclin-1 and LC3 were detected in hypertrophic, articular and end plate chondrocytes indicating that chondrocytes undergo autophagy. The role of hypoxia in the induction of autophagy was investigated by treating chondrocytes in normoxia/hypoxia with staurosporine. Cells in normoxia died but cells in hypoxia survived. In a hypoxic environment, cells express the transcription factor HIF-1α. Knockdown of HIF-1α in chondrocytes reduced expression of Beclin-1 suggesting that HIF-1α may be involved in regulation of autophagy. AMPK, a regulator of energy status, is also required for the induction of autophagy and is expressed by growth plate and end plate chondrocytes. Knockdown of AMPK allowed coimmunoprecipitation of Beclin-1 with Bcl2, an anti-apoptotic protein, reducing the level of free Bcl2 available to protect the cells from apoptogens. Furthermore, increased AMPK expression caused an inhibition of mTOR, a nutrient sensor and negative regulator of autophagy. Therefore, a low energy status activates AMPK and promotes autophagy, enhancing survival. Dr Shapiro also presented studies assessing the effect of osmolarity on cell function in the NP. The osmo-regulatory protein TonEBP is expressed at high levels in the intervertebral disc and kidney and protects NP cells from hypertonicity by promoting the expression of transporters and osmolytes. TonEBP silencing led to a loss of NP viability in hypertonic media indicating that its expression is required for survival in a hypertonic environment. Inhibition of TonEBP resulted in inhibition of Caspase 3 activity and apoptosis demonstrating that TonEBP inhibits apoptosis by blocking caspase 3 activation. The aggrecan promoter contains 2 TonE motifs for TonEBP binding and silencing of TonEBP or overexpression of a dominant negative version suppressed aggrecan promoter activity showing that TonEBP helps disc cells adapt to the hyperosmotic environment while also generating molecules that maintain the hyperosmotic mileu. Dr Shapiro concluded that induction of autophagy is reliant on HIF-1α and the expression of mTOR and AMPK; furthermore cells can sense their environment and generate signals to auto-regulate their function.

The second presentation of the session was from **Dr Nigel Loveridge** (University of Cambridge, UK) entitled 'Osteocytes: birth, life and death in a mineralised tissue'. Osteocytes function within bone as mechanosensors and detectors of matrix damage. There is also evidence to suggest that osteocytes control bone turnover and display an endocrine role with respect to phosphate metabolism. Dr Loveridge introduced the MLOA5 cell line which exhibits a late osteoblast phenotype capable of mineralising and associates with collagen fibrils. MLOA5 cells temporally express osteocytes selective markers including E11, MT1-MMP and MMP. Therefore, this cell line can be used to investigate the process of osteocytogenesis and how osteocytes adapt to surviving in a hostile environment. Dr Loveridge showed that osteocyte apoptosis is an important cue for targeted bone resorption. Osteocyte apoptosis can be caused by oestrogen deficiency, with oviariectomized rats displaying increased osteocytes apoptosis. Alterations in mechanical loading can also contribute to osteocyte apoptosis and disuse-induced bone hypoxia increases the expression of osteopontin *in vivo,* a protein that recruits osteoclasts. During bone loading, osteocytes release nitric oxide which inhibits bone resorption. Dr Loveridge provided evidence to suggest that osteocytes may control bone formation through expression of sclerostin and its effect on the Wnt signalling pathway. Sclerostin is an osteocyte specific protein that inhibits osteoblastogenesis by preventing canonical Wnt signalling. Sclerostin expression is turned off at sites of active bone formation and the distribution of sclerostin positive cells increases away from the bone surface. In loaded mouse ulna, sclerostin expression is switched off to allow new bone to form.

Apoptotic osteocytes are associated with sites of bone microdamage undergoing remodelling. Dr Loveridge therefore hypothesised that osteocyte derived factors might be associated with the bone changes observed in musculoskeletal diseases such as bone loss due to hip fracture or increased bone mass associated with hip osteoarthritis. In patients with hip fractures, the density of eNOS and nNOS positive osteocytes was decreased and a reduction in sclerostin positive osteocytes has been associated with hip OA. The presentation concluded with some questions for future research including 'Do osteoblasts/osteocytes regulate osteocytogenesis?' and 'Is the role of the osteocyte passive or active?'

New Investigator/Trainee session

The New Investigator/Trainee session began with **Dr John Williams** (Wellcome Trust, London, UK) talking about clinical/basic fellowship schemes from the Wellcome Trust. Dr Williams reflected on the new scientific disciplines developing and the modern facilities and equipment now available, commenting that this was an exciting time to be embarking on a scientific career. The Wellcome Trust is able to fund a new investigator throughout their careers through the different kinds of funding it provides. Dr Williams gave an overview of the application process, stressing the importance of developing a good idea which is key in prioritising the best applicants. He then summarised the ingredients for a good application including a strong and original hypothesis, a clear research plan, convincing pilot data, appropriate expertise, value for money and good communication. It was highlighted that an appropriate 'plan B' should be considered for the research plan if the proposed approach does not yield useful information. Dr Williams also emphasised the need to find the right supervisor and mentor and to build up a competitive CV.

The second presentation was from **Professor Karl Kadler** (University of Manchester, UK) comparing MRC training fellowships/fellowships with lecturer routes into academia. Professor Kadler also stressed the importance of finding a relevant question to drive research and highlighted that passion for a particular question will help researchers stay enthusiastic. A hypothetical time-line for a research career was presented, comparing the fellowship route with the lecturer route. Professor Kadler emphasised the need for an applicant to demonstrate their independence, through moving labs to learn new techniques, or by giving talks at other institutions, or through their role as corresponding author on published papers. The structure for a strong fellowship proposal was

recommended to involve independent career intentions, an important question, a good training environment at the host institution, and demonstration of collaborations outside of the lab and exploitations/applications/public engagement with science.

The third talk was from **Dr Keith Thompson** (University of Aberdeen, UK), an ARC funded research fellow. Dr Thompson completed his BSc, PhD and first post-doctoral position at the University of Aberdeen before being awarded an ARC travelling fellowship to visit Matthew Gillespie's laboratory in Melbourne, Australia. During this time, Dr Thompson applied for a fellowship with the ARC. From his experiences, Dr Thompson recommended discussing the research question with a supervisor/mentor, allowing plenty of time to improve the chance of success, obtaining feedback on the proposal from a number of different people and developing fairly thick skin! He also advised working on an interview technique that shows enthusiasm, clear communication, good body language and confidence rather than arrogance.

The final presentation of the session was from **Dr Vicky MacRae** (University of Edinburgh, UK), a BBSRC fellow and 2008 Barbara Mawer visiting fellowship recipient. Dr MacRae completed a BSc, PhD and two post-doctoral positions at the University of Edinburgh before applying for a BBSRC institute career path fellowship at the Roslin Institute, University of Edinburgh. A BBSRC institute career path fellowship provides funding for 5 years to early-career researchers wishing to be based at a BBSRC institute. Up to two institute career path fellowships are available per year and are targeted at researchers with 3-6years post-doctoral experience. Dr MacRae outlined the steps involved in preparation, writing of the proposal, and interviewing for the fellowship, highlighting the importance of displaying independence from current group, supervisory experience, nominating appropriate referees and showing ambition for the future with respect to research and grant opportunities. Dr MacRae was also awarded the 2008 Barbara Mawer visiting fellowship which allowed her to visit Dr Paul Genever in York and investigate the role of micro RNAs in bone mineralisation.

Monday:

Joint Symposium: New Frontiers for cartilage and bone research

The first talk was presented by **Professor Ian Clark** (University of East Anglia) with the subject "Micro RNAs and the skeleton". He provided a thorough introduction to micro RNA (miRNA) biology and the laboratory methods required to analyse these molecules. He then moved on to describe a screen of miRNAs expressed during the differentiation of the mouse chondrocytic cell line ATDC5. Among a number of miRNAs regulated during chondrogenic differentiation of this cell line he focussed on miR-140 and miR-455. miR-140 is found throughout the developing mouse skeleton and data was presented which implicated this miRNA in the regulation of the histone deacetylase HDAC4 through an interaction with the 3` untranslated region of the HDAC4 mRNA. Microarray analysis also suggested that miR-140 regulates expression of the gene CXCL12. Both miR-140 and miR-455 suppress SMAD dependent signalling in chondrocytes and, interestingly, TGFb-1 suppresses the expression of these miRNAs. It was therefore postulated that they play a role in how the cells respond to TGF or BMP signalling and that this could control the progression of chondrocyte hypertrophy.

The next speaker, **Dr Henry Roehl** (Sheffield University), provided a fascinating overview of the history and benefits of the zebrafish as a model system for developmental biology and demonstrated that it can be a powerful tool for examining skeletal biology. He showed how forward genetic screening has provided models of bone disease. One zebrafish mutant, called "Chihuahua", has a mutation in the COL1A1 gene which leads to an amino acid substitution identical to that which causes the human disease osteogenesis imperfecta. Another mutant creature lacks the EXT2 gene a heparan sulphate sulphotransferases and examination of the chondrocytes in these fish show that

they fail to grow along the axis of growth. This is of great interest as it resembles the pathology of the human multiple osteochondroma (MO) disease family which arises due to heterozygous mutations in EXT2 or the related EXT1 gene. Dr Roehl presented data showing that +/- EXT2 zebrafish cells could generally stack in the correct orientation but that they behave autonomously when close to the edge of a skeletal element. This leads to growth of cartilage in the wrong areas and sheds light on potential mechanisms of MO progression.

The final speaker in the "New Frontiers" session was **Professor Yuti Chernajovsky** (Queen Mary, University of London) who spoke about ex-vivo and in-vivo gene delivery strategies which could target matrix pathology in joints. He began by describing how a doxycycline-inducible dominant negative TNFa receptor introduced into mouse joints using a plasmid vector expression system could inhibit progression of collagen-induced arthritis. He then continued by remarking on how one of the greatest challenges in cytokine gene therapy is the targeting of the expressed protein to the tissue of interest. He showed how his lab had engineered a latent form of interferon by fusing it with the TGFb1 latency associated protein via a MMP cleavage site. This led to a long half life of the latent protein complex and activation of the cytokine only in tissues with MMP activity. Gene delivery of LAP-IFN using a plasmid vector resulted in significant inhibition of established collagen–induced arthritis. Importantly, introducing different cleavage sites within the MMP linker can make the activation of the cytokine specific to particular MMPS which will allow better targeting. Finally he presented data which used a similar technology to create "immunocytokines" where the cytokine and MMP site linker are attached to an antibody which specifically recognises collagen type II that has been modified by treatment with reactive oxygen species. This antibody binds to collagen type II in osteoarthritic cartilage and not in normal cartilage and will allow gene delivery of proteins targeted to osteoarthritic cartilage.

Joint Symposium: The control of angiogenesis in bone and cartilage

The first talk in this next session was presented by **Dr Marie-Hélène Lafage-Proust** (Saint-Etienne University). She described how, using micro CT scanning and histological analysis, blood vessels and capillaries could be visualised in bone and that there is a tight relationship between blood vessel growth and bone formation. She showed that, in a rat model, treadmill exercise induced both bone mass and blood vessel formation whilst limb immobilisation led to decreased bone formation and reduced the bone blood vessel network. Therefore mechanical stimulation led to highly coupled responses of both bone remodelling and blood vessel formation and she presented data implicating VEGF in this process. However this close link between enhanced bone mass and blood vessel formation which resulted from different loading regimes was not always observed when bone was exposed to other stimuli. Examination of hypoxic stimulation did not lead to increased bone mass and actually led to an impairment of osteoblast function, but bone angiogenesis was nevertheless stimulated. Conversely, parathyroid hormone stimulation increased bone mass but reduced the number of blood vessels. Therefore, the relationship between bone formation and angiogenesis is a complex one.

The final speaker before lunch was **Professor Tom Clemens** (University of Alabama) who presented work aimed at understanding how targeting of hypoxia inducible factor (HIF) pathways could be used to control bone repair. He described how angiogenesis was essential for delivery of oxygen to developing and regenerating bone and how the HIF transcription factors are responsible. He presented data from mouse model systems which illustrated this. The first system was a transgenic animal harbouring an osteoblast-specific knockout of the *Vhl* gene, which encodes a protein that is critically involved in HIF1α degradation. In effect, this mouse produces an osteoblast specific HIF1α over expressing phenotype and exhibits significant increased bone density in both

cortical and trabecular bone. This phenotype was associated with increased angiogenesis and upregulation in VEGF signalling. The opposite was observed in mice that contained an osteoblast-specific conditional knockout of HIF1α. These mice had thinner bones and reduced angiogenesis compared to controls. Interestingly analysis of *in vitro* cultured metatarsals revealed an increase in endothelial sprouting in *Vhl -/-* cultures but isolated osteoblasts from these mice are not significantly impaired. This indicates that the HIF1a pathway is involved in bone development and remodelling, acting in a cell non-autonomous manner.

Dr Ann Canfield commenced the session with her talk entitled: **Molecular regulation of vascular calcification**. Vascular calcification is an independent risk factor for cardiac events and mortality, and has been shown to involve osteo- and chondrogenic differentiation of vascular smooth muscle cells and pericytes present in the vessel wall. Dr Canfield's presented work that focused on understanding how the fate of vascular progenitor cells is regulated and thereby identify therapeutic strategies for targeting vascular calcification. Data was discussed on the role of bisphosphonates in regulating vascular calcification. In a model of vascular calcification using nephrectomised rats, zoledronate and etidronate inhibited vascular calcification *in vivo*. Farnesyl pyrophosphate prevented the inhibitory effect of zoledronate, but not etidronate, suggesting that selective effects of vascular calcification could be manipulated by the use of bisphosphonates.

Short Oral Presentations

Matrix mineralization: a driver for osteocytogenesis? OC1. Matt Prideaux discussed his efforts in understanding the factors driving osteocytogenesis. Since only approximately 20% of osteoblasts go on to differentiate into osteocytes, the process which leads to loss of these cell numbers and cell processes is important in osteocyte maturation. Use of the MLO-A5 cell line to analyse the processes involved was discussed. Cell cultures treated with ascorbic acid and phosphate showed increased expression of osteocyte markers, including E11 and sclerostin. His work found a primary role for mineralization in osteocytogenesis, since cultures lacking phosphate or containing sodium pyrophosphate, to inhibit mineralization, resulted in decreases in osteocytic differentiation. He also reported that osteocyte differentiation can be re-induced in cultures where mineralization is initially inhibited and then restored by removal of inhibitors. These findings suggest an important role for ECM mineralization in terminal osteoblast-to-osteocyte differentiation.

Endochondral osteoblasts OC2. Chrissie Hammond discussed the role of hedgehog signalling in endochondral osteoblasts. She described a zebrafish model in which bone development was studied *in vivo*. Their group have identified two populations of osteoblasts with different sensitivities to Hedgehog signalling. One population of osteoblasts was found outside cartilage and required a low level of Hedgehog signalling. A second population of osteoblasts arose from transdifferentiation of chondrocytes and required a higher level of signalling for their formation. They also found that Hedgehog signalling is required for proper vertebral patterning and the formation of neural and haemal arches, which are the zebrafish equivalents of amniote ribs

Hypoxia and HIF in Ewing's sarcoma OC4. Hypoxia is known to be a feature of solid tumours and gene expression is regulated via stabilisation of the Hypoxia-Inducible Factor (HIF). HIF-induced genes modulate pathways central to cell survival and expansion in an oxygen-deficient environment. Less is known about HIF expression in primary bone sarcomas. **HJ Knowles** described HIF expression in Ewing's sarcoma. Of 53 Ewing's tumours analysed, 28% were positive for HIF-1alpha, 26% for HIF-2alpha and 19% for the two molecules together. HIF was found adjacent to areas of necrosis in Ewing's sarcoma specimens. The authors proposed a role for hypoxia in the *in vivo* induction of HIF and suggest it plays a role in the pathobiology of Ewing's sarcoma.

Osteogenesis and HUVECS OC3. JM Kanczler discussed the importance of the microvascular endothelium in the development, growth and fracture repair of bone, during which processes endothelial cells play an essential role. In their experiments, HUVECs were co-cultured with foetal or adult human bone marrow stromal cells. Using real-time PCR, co-cultures were analysed for alkaline phosphatase (AP) and type-1 collagen expression. AP mRNA expression was elevated in co-cultures with HUVECS and adult stromal cells compared with cultures comprising only of single cell types. Type I collagen was also increased in co-cultures of adult and foetal co-culture experiments, suggesting that osteoprogenitor-endothelial interaction is required to enable the vascularisation and regeneration of bone in clinically relevant conditions such as delayed and non-union bone fractures

HDACs as chondroprotective agents PO86. Cartilage destruction is primarily believed to be mediated by the MMPs (Matrix metalloproteinases) and ADAMTSs (a disintegrin and metalloproteinase with thrombospondin type I motifs). MMP and ADAMTS expression can be altered by protein acetylation that is mediated by histone acetyltransferases (HATs) and HDACs (histone deacetylases). **KL Culley** discussed data showing how three specific HDAC inhibitors are chondroprotective in experiments performed on a chondrcoyte cell line. The effects of trichostatin A (TSA), valproic acid (VPA) and MS-275 were investigated in cytokine-stimulated chondrocyte cultures. Results showed a reduction in MMP-1 and MMP-13 expression by RT-PCR by TSA and VPA but not MS-275. All three compounds reduced MMP-3 expression. Although all three compounds work slightly differently in inhibiting HDACs, they all protect against cytokine induced collagenase MMP-3 expression, suggesting this might be their mechanism of chondroprotection.

MIR-675 and collagen II levels PO91. MicroRNAs are endogenous RNAs that have recently emerged as gene-regulatory molecules. Although they play a regulatory role in several biological processes, their role in cartilage is not understood. **K Dudek** described how previous work by their group has shown that H19, a non-protein-coding RNA, has similar expression to the main cartilage matrix genes including type II collagen and aggrecan. H19 has been found as a primary miRNA transcript, miR-675, whose function was investigated. Using RNA interference experiments, they found that miR-675 increased Col2A1 levels at the mRNA and protein levels. They suggest that miR-675 may be regulating Col2a1 expression to maintain the differentiated chondrocyte phenotype.

Chondroitin sulphation PO19. S Mukhopadhyay described the use of monoclonal antibodies for isolating chondrocytes from arthritic cartilage samples. Pronase and collagenase treated chondrocytes were harvested and fluorescently labelled with monoclonal antibodies 3B3, 7D4, 4C3 and analysed by flow cytometry. Analysis of the isolated cells by FACS analysis showed subpopulations of chondrocytes could be identified by the specific antibodies and future work aims to identify potential stem/progenitor cells. Further studies are planned which will aim to sort cells into stem/progenitor cells using the specific antibodies and use them in new generation ACT technologies.

ADAMTS-4 and VEGF PO74. Angiogenesis is key to the stimulation of endothelial cell proliferation, migration and tube formation. VEGF (vascular endothelial growth factor) mediates its effects by binding to receptor kinases VEGF R1/R2. ADAMTS-1 and ADAMTS-4 are two aggrecanases implicated in angiogenesis. ADAMTS-1 has been shown to be anti-angiogenic by binding to ADAMTS-1. ADAMTS-1 and ADAMTS-4 can also bind heparin, and heparin sulphate may modulate their activity and localisation. ADAMTS-1, ADAMTS-4, VEGF, VEGFR2 and NP1 were combined to evaluate their formation of complexes. Western blotting was used to detect the proteins. ADAMTS-1 and ADAMST-4 bound to VEGF and NP1. These interactions occurred both in the presence and absence of heparin. Neither ADAMTSs bound to VEGFR2. These data suggest new mechanisms regulating angiogenesis via ADAMTS-1, ADAMTS-4 and VEGF. ADAMTS-4 inhibited tubule

formation in the presence and absence of VEGF and the authors propose that it is therefore a potential anti-angiogenic target.

Knockout of P581PK PO82. SJ Gilbert described data from a study showing that P581PK-/- mouse. P581PK is an inhibitor of PKR (a TNF activated protein kinase). In studies using these knockout mice, P581PK-/- mice developed more severe cartilage damage than wild-type littermates. These results suggest a role for PKR in arthritis and offer a model for studying signalling pathways in arthritic disease.

Apoptosis in anteromedial gonarthrosis PO81. Apoptosis is known to occur in OA, but the mechanisms underlying its activation are not fully understood. Cell death was analysed in human osteoarthritic samples with anteromedial gonarthrosis. There was a high level of chondrocyte apoptosis in the region of this pathology, and it was suggested that mitochondrial pathway and reactive oxygen species (ROS) are involved by activation of caspase 3 and cytochrome C.

Imaging and RAMAN PO80. Jay Dudhia described the use of Raman spectroscopy in the assessment of cartilage damage using specimens of normal and arthritic cartilage from human donors. The technology obtains spectra from arthritic lesions using a laser spectrometer diode. Spectra obtained from cartilage were correlated with tissue sections spectroscopy was able to measure changes in sulphation (SO_4^{2-}), tissue hydration and collagen content. The author proposed that this methodology is cheaper than MRI (magnetic resonance imaging) and is portable, which may have important implications for clinical applications.

CTGF and lung fibrosis PO33. M Ponticos described a role for CTGF (connective tissue growth factor) in the promotion of lung fibrosis. Bleomycin-treated transgenic mice carrying the Col1a2 and CTGF promoter/reporter genes were used. They found that Col1a2 expression/promoter activity in mice given a bleomycin challenge was proceeded by CTGF promoter activity. The lung fibrotic phenotype was also found to be mediated by MAP Kinases.

Wiskott-Aldrich syndrome PO31. Brit Wolters discussed the role of WAVE 1 (Wiskott-Aldrich syndrome protein verprolin homologous 1) in promoting cellular invasion by MT1-MMP (membrane-type 1 matrix metalloproteinase). MT1-MMP is localised to the leading edge in migrating cells, and WAVE proteins are thought to play an important role in this process. They found that the localisation of MT1-MMP at the leading edge of migrating cells is dependent on WAVE1-mediated organisation of the cytoskeleton. This may have significant therapeutic implications for the cellular invasion of tissue in diseases including cancer and rheumatoid arthritis.

Joint Symposium: Dent (BRS) and Fell-Muir (BSMB) Lectures

The last two presentations of the afternoon involved lectures given by leaders in the fields of bone and cartilage biology. **Professor Alan Boyde gave the first ever 'Dent lecture'** which focused on developments in bone biology over his career. Highlights of developments in the field were described, including the recognition of the contribution of the work of Charles Dent in the field of osteoporosis and osteomalacia. More recently new imaging modalities which have led to an improved understanding of bone biology were discussed e.g. scanning electron micrographs to analyse the mechanism of prosthetic implant failure and the investigation of the role of specific cell types including adipocytes in bone biology.

Following this, **Professor Tim Hardingham delivered the Muir-Fell lecture**. Professor Hardingham's lecture was a philosophical look back over his career which discussed the importance of proving and disproving the many uncertainties in science. He described his pioneering work on the structure and function of hyaluronan. He also discussed how a major challenge for the future will

be how to interpret the large amount of information scientists are now able to acquire within the field of matrix biology and how all this information can be integrated to improve our understanding of biology and pathology.

Tuesday:

Joint Symposium: Skeletal Development

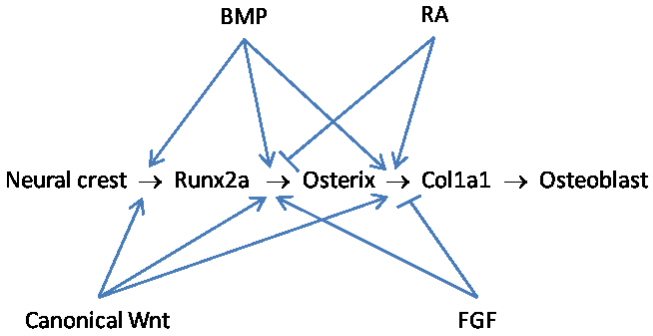
The final day of the conference began with the Joint symposium discussing skeletal development, chaired by Irving Shapiro (Philadelphia, USA) and Vic Duance (Cardiff, UK). Three invited speakers; **Bjorn Olsen** (Boston, USA), **Stefan Mundlos** (Berlin, Germany) and **Henry Roehl** (Sheffield, UK) gave elegant and engaging talks raising the importance of understanding morphogenic determinants, bone dysplasias and the examination of both dermal and endochondral ossification pathways of osteoblast differentiation.

Bjorn Olsen (Boston, USA), Professor of Developmental Biology/Harvard School of Dental Medicine opened his talk (**IS14)** by highlighting the types of experimentation used for gaining insights into skeletal development, such as knock-out mice, and examining human gene-linked diseases. He said that although these experiments are invaluable for understanding signalling mechanisms, 'cytokines and transcription factors were not enough'. Furthermore, simply having the right cell in the right place was not sufficient to explain skeletal development and patterning. Professor Olsen said 'there was not enough known about architecture'. Polarised structures, distinct shapes that develop before tissues are formed – these are the defining factors of architecture. Skeletal morphogenesis needs mechanisms to control cell and tissue polarity. The primary cilium is just that - a mechanism for polarising the cellular structure of skeletal cells required for skeletal architecture. Olsen described osteoblast and chondrocyte primary cilium as 'beautiful' and went on to discuss their importance in more detail. Polycystin-1 (Pkd-1) together with polycystin-2 is a mechanosensor and signal transducing element in renal epithelial cells. They are associated with the primary cilium. The question was asked whether Pkd-1 is a mechanosensor, or part of a transducing complex in cartilage and bone? Olsen described the use of *Wnt1*-cre mice to conditionally remove polycystin-1 from the majority of skeletal tissues its effect. Olsen showed that conditional inactivation of *Pkd-1* in mesodermal cells decreased growth of the posterior skull, decreased proliferation and increased apoptosis in the presphenoid synchondrosis. Delayed ossification lead to decreased growth of the upper part of the nasal bone and the frontal-nasal suture was compromised. It was therefore concluded that Polycystin-1/2 is critical for craniofacial development, growth, and response to mechanical input.

The second talk (**IS15**) entitled 'Bone development and dysplasias', was given by **Stefan Mundlos** (Berlin, Germany). He began by explaining how Hox genes are important in limb and spine development in mammals. Mutations within HOX genes can lead to limb malformations, such as the synpolydactyly (SPD) phenotype caused by poly-alanine extensions in HOXD13 gene. Mundlos used mouse mutant synpolydactly homologue (spdh), which has extra digits with fusions, to explore the molecular pathogenesis of synpolydactyly. Results showed retinaldehyde dehydrogenase-2 (Raldh2), the rate limiting enzyme for retinoic acid (RA) synthesis, was down-regulated in the limbs of spdh/spdh mice. Raldh2 is co-expressed and directly regulated by Hoxd13. The Raldh2 reduction correlated with a reduction in RA expression in the mice. Intrauterine treatment with RA restored the pentadactyly in the spdh/spdh mice. Through siRNA knockdown of RA they demonstrated that RA inhibits chondrogenesis of mesenchymal progenitor cells, as does Hoxd13. However, Hoxd13 with expanded Ala repeat promotes cartilage formation, which is associated with increased sox6/9 expression. The spdh/spdh mice limbs have increased sox9 expression and ectopic cartilage in the interdigital mesenchyme, suggesting uncontrolled mesenchymal to chondrogenic differentiation. Mundlos concluded that polydactyly in SPD is caused by increased interdigital chondrogenesis as a

result of reduced RA levels by the prochondrogenic effect of the mutated Hoxd13 gene. Therefore Hox genes pattern the developing limb through the regulation of cell differentiation and proliferation.

Henry Roehl (Sheffield, UK) discussed 'osteoblast differentiation is regulated by multiple signalling pathways in the zebrafish embryo' (**IS16**). Roehl presented a time course of osteoblast markers to further our understanding of cranial bone development. To achieve this they used the zebrafish model and in particular, followed the development of the Opercle (the bone that forms the gill cover). In general, three stages of development were identified where 1) runx2a expression was initially quite diffuse. Its expression increased over time, becoming more specific to the skeleton. It was switched off before 120 hours post-fertilisation. 2) Osterix was expressed at a later time point and remained more specific to the skeleton than runx2a. Its expression peaked and fell before 120hours.



3) Collagen 1a2 was co-expressed or followed osterix expression. Roehl investigated the impact of chemical treatment and heat shock promoters to dissect this pathway. BMP and retinoic acid (RA) affected runx2, whereas BMP, WNT and FGF affected osterix expression. BMP, RA and FGF influenced mature osteoblasts. RA signalling on this pathway and showed that early treatment decreased dermal bone ossification. RA impacted on runx2b expression rapidly and the use of DEAB, an RA inhibitor reversed the effect although over a longer time period. Osterix responded more slowly (4hours compared to 2hours for runx2b). Furthermore, inhibition of RA synthesis for 12hours increased bone formation. RA treatment had no other noticeable effect on other neural crest derivatives such as pig cells, myocytes, glia, neurons and chondrocytes, with the exception of adipocytes. Since the skull forms by both endochondral ossification and dermal ossification, this model was proposed as an interesting *in vivo* model of osteoblast recruitment and maturation.

BSMB Open Session

The first talk (**P064**) entitled 'FGF receptor-dependent, integrin-independent phosphorylation of P38, JNK and ERK MAPKs following mechanical stimulation of human articular chondrocytes in primary monolayer cell culture' covered the results of **Y Zhou** (Edinburgh, UK) but was presented by G Nuki. Nuki explained that mitogen activated kinases are involved in many inflammatory and stress responses. Previously published data demonstrated Jun N-terminal kinase (JNK) caused an increase in proteoglycan synthesis of mechanical stimulated (MS) human chondrocytes, which was thought to be partially integrin dependent. The project aim was to determine if phosphorylation of MAPKs in normal and osteoarthritic (OA) human articular chondrocytes (HACs), following cyclic mechanical stimulation, was integrin and/or FGF-2 receptor dependent. The cells underwent MS in the presence or absence of gadolinium, FGF receptor inhibitor (PD173074), integrin blocking antibodies for beta1 (P4C10 and JB1A), alpha5 (BIIG2) and alphavbeta5. Proteins were analysed via immunoblotting. P38, ERK 1/2 and JNK were identified in both populations of HACs. Phosphorylation of p38 increased in both cell populations after 5 minutes MS. ERK 1/2 phosphorylation in normal chondrocytes increased after 10 minutes MS, but more variable in OA HACs. Normal and OA HACs showed increased 46kDa JNK phosphorylation after 10 minutes but no increased 54kDa JNK phosphorylation. Pre-incubation of cells with integrin blocking antibodies and gadolinium demonstrated no inhibition of MS induced MAPK phosphorylation. MS induced p38 and ERK phosphorylation was inhibited by FGF receptor inhibitor. Nuki concluded that both normal and OA HACs express MAPKs in primary monolayer culture and p38, JNK and ERK1/2 are

phosphorylated in response to MS. Finally MAPKs activation in these cultures was FGF receptor dependent and not mediated by integrins or stretch activated ion channels.

Tonya Vincent (London, UK) presented the work of **C McLean** who used confocal microscopy and electron microscopy to examine components of the pericellular matrix in skeletally immature, mature and osteoarthritic cartilage (**P034).** The confocal microscopy in mature cartilage showed strong staining for collagen VI and heparin-sulphated perlecan, whose expressions were close, but not wholly co-located. Co-localisation of FGF-2, which binds perlecan in the pericellular matrix was seen, as was shown previously. Aggrecan and type II collagen were not detected in the pericellular matrix. Link protein was seen at the outmost aspect of the chondron and extended towards the territorial matrix. This specific banding was not seen in young cartilage and link protein was detected throughout the pericellular and territorial matrices, as was aggrecan. Perlecan, type VI collagen, FGF2, and CTGF were all increased in the osteoarthritic pericellular matrix. A proteomic analysis of porcine chondrons was presented. The pericellular matrix was dissociated and the cellular proteins extracted and analysed. Analyses of these fractions did not detect perlecan or FGF-2. These were probably too big or small for the crude method used, however, other known proteins of the pericellular matrix were detected that included type XI and type IV collagens and biglycan. As predicted type II collagen and aggrecan were not detected in the pericellular matrix. There were distinct differences in the matrix components of young and mature or osteoarthritic pericellular matrices. It was proposed that the younger cartilage was still developing or was more synthetic. Perlecan-bound growth factors in the pericellular matrix were evidence for the role of this region in harbouring and releasing regulatory factors.
The next talk (**P021)** entitled 'Expansion on a hyaluronan coated surface enhances the chondrogenic potential of human mesenchymal stem cells' was given by **C. Ryan** (Galaway, Eire). Ryan described how hyaluronan (HA) treatment, via inter-articular injection, is used as a mode of pain relief for osteoarthritic (OA) patients. HA is a major component of the synovial fluid and cartilage matrix, but its mode of action in pain relief is unknown. To test HA as a cartilage regenerative agent its effect on chondrogenic differentiation of mesenchymal stem cells was tested *in vitro*. Human mesenchymal stem cells (hMSCs) were isolated from bone marrow and plated onto cell culture surfaces coated with either 1mg/ml HA-A (Supartz), HA-B (Duraline) or chondroitin sulphate. hMSCs were also cultured in soluble forms of HA-A and HA-B. After 7 days in culture chondrogenic markers sox9, aggrecan (Agg) and collagen type II (Colll) were measured via real-time PCR. Cells exposed to HA for 7 days were induced toward chondrogenesis by pellet culture and 10ng/ml TGFβ3, followed by glycosaminoglycan (GAG) content analysis via Safranin O staining and BMMB assay quantification on day 21. Ryan described how cell expansion on HA-A and HA-B increased Sox9 expression 2.5-fold and 5.8-fold respectively. Cells expanded on HA-B demonstrated a 2.4-fold increase in Agg and 4.8-fold increase in Colll expression, but cells expanded on HA-A demonstrated no change. The hMSCs cultured in soluble HA demonstrated no significant changes in chondrogenic marker expression. Cells expanded on chondroitin sulphate showed increased GAG expression, but both Agg and Colll expression down-regulated. Pellet cultures expanded on HA showed increased GAG/DNA ratio, which was unchanged in other treatment groups. The talk concluded that expansion of hMSCs on HA coated surfaces increases their chondrogenic potential and could potentially be used as a tool in clinical cartilage regeneration.

N Eltawil (London, UK) presented detailed *in vivo* data examining the molecular response to cartilage injury (**P066**). Joint surface repair mechanisms require further study since their outcomes can lead to post–traumatic osteoarthritis or relatively good repair. They used C57BL/6 and DBA/1 mice to assess repair responses to full thickness injuries comparing these to sham and non-operated joints. Repair responses were measured using histological scoring. Molecular responses were identified and measured using real time PCR and immunohistological techniques. Their results showed strain dependent responses where the DBA/1 mice were able to repair lesions more

effectively and the C57BL/6 mice developed post-traumatic osteoarthritis. FRZB expression was decreased and axin2 mRNA expression was increased in both strains showing an activation of Wnt signalling in these models of cartilage damage. In particular Wnt16 was up regulated with injury and this was more pronounced in the C57BL/6 strain. They suggested these models would be useful for further studies of cartilage injury and repair.

L.Ramage (Edinburgh, UK) presented a talk (**P088)** entitled 'Involvement of NMDAR in chondrocyte cell death and matrix degeneration'. She described how NMDA receptor is expressed on chondrocytes but its function is unknown. The receptor is commonly expressed in neurones and its over-stimulation results in cell death and neuronal degeneration. To understand the role of the receptor in chondrocyte cell survival and matrix homeostasis rat femoral heads were treated with NMDA (50μM or 1mM) for 72 hours followed by fixing and staining for TUNEL and live/dead assays. Ramage found that excessive stimulation of the NMDA receptor resulted in increased cell death and proteoglycan loss from the femoral heads. Also normal and osteoarthritic human articular chondrocytes (HACs) were cultured in monolayer and then treated with 50μM NMDA for up to 24 hours, followed by RNA extraction for later qRT-PCR analysis of matrix and collagenase genes. RT-PCR showed that NMDA treatment caused a decrease in *MMP*3 and increase in TIMP1 expression in normal HACs, with aggrecan, collagen type II and *MMP*13 unaffected. OA HACs treated with NMDA had reduced collagen type II, *MMP*13 and TIMP1 expression, with aggrecan and *MMP*3 unaffected. She concluded that the stimulation of the NMDAR has the potential to modify cartilage structure, shown by the induction of cell death and reduced proteoglycan expression in stimulated normal cartilage. She also stated there appears to be a shift in anabolic (↓ *MMP*3, ↑ TIMP1) to catabolic (↓ collagen and TIMP1) gene expression in NDMA stimulated OA cartilage.

WS Khan (Manchester, UK) discussed the findings of mesenchymal stem cells isolated from the synovial fat pad in the terms of their differentiation potential cell surface markers (**P022)**. These cells have osteogenic and chondrogenic differentiation potential that remain to be fully characterised. Clonal populations were isolated and cultured in parallel with mixed populations, and their stem cell surface epitopes identified. These cells were then cultured in differentiation medium (osteogenic and chondrogenic), where those in chondrogenic medium were grown as cell aggregates. All cell populations expressed markers for adult mesenchymal stem cells. Of the six clonal populations, two showed stronger osteogenic responses and one showed a stronger chondrogenic response when compared to the mixed populations. Their DNA and GAG content were comparable. The clonal populations showing osteogenic or chondrogenic differentiation potentials were proposed to be inherently different. Identification of this inherent property was suggested to be useful for identifying stem cells with differentiation potential from the synovial fat pad.

E. Jones (Norwich, UK) gave a presentation (**P060)** on 'Mechanical strain modulation of matrix metalloproteinases in human tenocytes'. She opened the talk describing tendinopathies as a range of diseases that cause severe morbidity, which are associated with risk factors mechanical strain and dysregulated matrix metalloproteinase (MMP) expression. The project aim was to investigate the effect of mechanical strain (MS) on MMP expression. To explore this 3D collagen gels were seeded with human Achilles tenocytes then stretched (using a sinusoidal waveform of 0-5% at 1Hz) using a flexcell system. Two cell densities were tested, $0.5x10^6$ and $1.5x10^6$ cells/ml. The media and cells from the gels were then harvested and analysed for changes in gene expression via qRT-PCR. Time course experiments from 0-48 hours showed that the effect of MS on tenocyte *MMP* expression varies possibly due to cell density. At the lower cell density there was no significant change in gene expression of *MMP1*, *MMP2*, *MMP3*, *MMP13* or *MMP23* during the 48 hour time course. MMP1 expression was significantly decreased in the higher cell density after 48 hours.

There was no significant change in ADAM12 expression at either cell density. The expression of elastin and fibulin in response to MS was also tested at the lower cell density and a significant increase in elastin expression was seen at 8 hours. There was no significant change in fibulin expression. Jones concluded that MS may play an important role in tendinopathy development, indicated by the modification in *MMP* expression of tenocytes in response to biologically relevant levels of strain. She also stated that the differences in *MMP* expression seen between the two cell densities, in response to strain, could indicate that cell-cell interactions are important in influencing cellular responses to strain.

B Poulet (London, UK) examined the contribution of genetic versus mechanical influences on the aetiology of osteoarthritis (**P065)**. This was carried out by using a mechanical loading model in CBA mice compared to Str/ort mice, which develop spontaneous osteoarthritis. CBA mice knee joints were subjected to mechanical load 3 times per week over two weeks. Cartilage lesions were examined and compared to contra-lateral non-loaded knees. Mechanical loading increased cartilage lesion scores, increased the collagen type II degradation marker and decreased UDGH (a marker for glycosaminoglycan synthesis). Proteoglycan loss, as measured by safranin-O staining, was highly localised to the lesions. These findings largely reflected that seen in the Str/ort mouse, age-matched strain, although the location differed. The lesions were primarily seen in the lateral femur of the CBA mice, whereas lesions were primarily found in the tibial plateau of the Str/ort mice. Further examination of this model was proposed and future experiments may include a rest period following loading to assess lesion progression without loading.

BSMB Matricellular proteins in health and disease

Chaired by David Abraham and Hideaki Nagase, the first talk of the session (**IS19)** was given by **Karen Lyons** (Los Angeles, USA) was entitled 'The roles of connective tissue growth factor and CYR61 in skeletal development'. The talk explored the function of CCN family protein CCN2, also known as connective tissue growth factor (CTGF). She introduced the CCN proteins as a family of multi-cellular proteins that impact on cell adhesion, migration and other cellular functions through their interaction and activation of integrins. CCN proteins can also bind a range of growth factors and interact with and modify matrix metalloproteinase activity. CCN2 is known to regulate cartilage ECM production via integrin-mediated signalling. They previously found that CCN2 is required for various stages of chondrogenesis, such as growth plate angiogenesis via the up-regulation of VEGF expression in hypertrophic chondrocytes. Transgenic $CCN2^{-/-}$ mice were used to explore the function of the gene further. They found that the mice died at birth and showed generalised chondroplasia. Further analysis indicated reduced safranin O staining, aggrecan and link protein, but no significant change in collagen content. The bones of the mice were bendy and phenotypically similar to that of aggrecan deficient mice. The mutant nucleus pulposus was then examined, which normally has CCN2 expression and high aggrecan content. The $CCN2^{-/-}$ mice had proteoglycan deficiency and defective intervertebral disc formation. Reduced levels of alpha-5 were found, which is interesting as CCN2 is a known integrin α5ß1 ligand. The peri-cellular matrix in this region was also abnormal with shorter and thinner collagen fibres. The proliferating chondrocytes had enlarged endoplasmic reticulum (ER) and large vacuoles containing ECM components, attributed to the cells having abnormal ECM secretion. The large ER correlated with increased expression of protein-folding chaperone BIP. This suggests that CCN2 has a role as an intracellular chaperone for ECM component export. Laser capture and micro-array identified that most genes are down-regulated in the mutant, apart from ER stress genes which had increased expression. She postulated that down-regulation in gene expression was due to cells trying to stop the transcription and translation of proteins due to their impaired secretion pathway. The vascular basement membrane of these mice was impaired due to abnormal ECM. CCN2 in hypertrophic chondrocytes is activated by Wnt signalling, and is repressed in proliferating chondrocytes by Sox9. Through transgenic mice and immunoprecipitation they have identified a region in the CCN2 promoter that is sensitive to Sox9, ß-

catenin and Runx2. Conditional murine knockouts for CCN2 and CCN1 have identified overlapping functions of the genes in chondrogenesis and osteogenesis. She concluded by saying that the CCN2/CCN1 double mutants exhibit profound chondroplasia, due to the genes' overlapping functions in chondrocyte proliferation.

Mats Paulson (Cologne, Germany) discussed the roles of matrilins and COMP in cartilage assembly, and in the pathogenesis of chonrodysplasias (**IS20)**. An overview of perifibrillar proteins and their role in regulating the assembly and interaction of matrix fibres was given, focussing on COMP and matrilin-3. The domain structures of the matrilins and COMP were shown and an explanation of their interactions and that of collagen IX. A beautiful gold-labelled EM image generated by Paulson's collaborators showed a native extract of collagen VI. This revealed matrilin to be attached to decorin and biglycan near the surface. This was further evidence for matrilin as an adaptor protein mediating matrix interactions. Collagen IX was said to be a major anchoring protein for COMP and matrilins since mice lacking collagen IX showed decreased COMP and matrilin. Single and multiple knockout mouse models of matrilin proteins did not generate significant phenotypes. In fact the only phenotype seen was that of increased collagen I density in the matn1$^{-/-}$, matn3$^{-/-}$ double knockout. The importance of COMP in collagen fibre assembly *in vitro* was highlighted. In the collagen IX$^{-/-}$ model, collagen fibril diameter was increased. However, in the double collagen IX/COMP knockout model, a normalisation of fibril diameter was seen, suggesting that COMP counters the collagen IX$^{-/-}$ phenotype. Certain mutations seen in COMP resulted in an altered ability to secrete the protein and cause disease. Consequently mutated/misfolded proteins were retained leading to ER stress. Several COMP mutations were analysed and a variety of affects on matrix formation were found, such as mice expressing COMP mutations showed a characteristic light area around the chondrocyte. However, not all mutated proteins were retained. Some mutated proteins could still be secreted and cause disease.

BSMB Young Investigators Award

The winner of the BSMB Young Investigators Award was **Jerome Lafont** (London, UK) gave an excellent talk on the responses of chondrocytes to hypoxia. He described how chondrocytes cultured *in vitro* differentiate into a fibroblast like phenotype, associated with reduced SOX9, aggrecan and collagen type II expression and increased collagen type I expression. Hypoxia is a strong promoter of the chondrocyte phenotype, which they tested by growing primary human chondrocytes in monolayer in 1% O_2. Though the use of western blotting and immunohistochemistry they found hypoxic conditions resulted in increased collagen type II and Aggrecan expression. Western blotting showed SOX9 was increased in 1% O_2, but with 20% O_2 SOX9 expression was no longer seen. The siRNA knockdown of hypoxia inducing factors HIF1 and HIF2, within primary chondrocytes, indicated HIF2α controls the expression of SOX9 and collagen type II. Lafont postulated increased HIF2 upregulates SOX9 expression which goes on to activate the expression of collagen matrix specific genes. He then spoke about prolyl hydroxylase-containing proteins (PHDs) which catalyse HIF prolyl hydroxylation resulting in their labelling for proteosomal degradation. LaFont postulated that inhibition of PHD proteins by a non selective dioxygenase DMOG (dimethoxalygycine) could result in increased HIF expression and a maintained chondrocyte phenotype. However, they found that DMOG caused problems in collagen type II secretion, thus a more specific PHD inhibitor would have to be developed. They previously found H19, a non-coding RNA which functions as a primary microRNA, to be highly expressed and hypoxia-inducible in primary chondrocytes. Using qRT-PCR based techniques they found H19 expression levels were comparable with collagen type II and aggrecan expression. Through siRNA they identified H19 inducible though HIF2α. H19 hypoxic induction was largely abolished by depletion of transcription factor SOX9. They also found MiR-675, the mature microRNA which is derived from H19, was also hypoxia-inducible and SOX9-dependent. The depletion of MiR-675 reduced hypoxic induction of cartilage matrix gene, Col2a1. He concluded that miR-675 is SOX9-dependent and regulates

expression of the most important cartilage matrix gene, Col2a1. Therefore this potentially represents a new mechanism for regulating cartilage matrix expression.

Musculoskeletal Research Opportunities.

Mark Pitman from the MRC gave an informative talk about the funding opportunities available from the MRC and how to best attain these grants. He outlined the structure of the MRC institution and explained its remit, which was to 'improve human health'. The MRC has published its new 2009-2014 strategic plan. It has four main strategic aims: 1) Picking research that delivers: Setting research priorities that are most likely to deliver improved health outcomes. I) Resilience, repair and replacement, II) Live a long and healthy life, 2) Research to people: Bringing the benefits of excellent research to all sections of society, 3) Going global: Accelerating progress in international health research and 4) Supporting scientists: Sustaining a robust and flourishing environment for world-class medical research. Mark Pitman said the MRC has a budget of £1.6million per year that and despite commonly held belief – does fund basic research. He suggested signing up for their email updates and visiting their website regularly. One of the more hard hitting messages was that 'in the past, proposals coming from the musculoskeletal disease research field were not up to the standards required' and he claimed that this had contributed to the widely-held belief that MRC did not look upon funding basic research favourably. Thankfully MRC acknowledges that this is no longer the case, and our field provides good quality competition for disease areas such as cancer and cardiovascular diseases. Key tips for writing proposals were to write clear, focussed proposals and to pay heed to the costing aspect. The take-home message was broadly: 'If the proposal was good enough, MRC will fund it'.

Joint Oral Posters

The elastic network in human articular cartilage: an immunohistochemical study of elastin fibres and microfibrils. **J Yu** (Oxford, UK). Yu (**P098**) described how cartilage biopsies were taken from the trochlear groove and medial and lateral femoral chondyle regions of healthy human cadaveric knees to study elastin and microfribril networks within articular cartilage. Dual fluorescent immunostaining was completed for elastin and fibrillin-1 (fibrillin-1 is a major component of microfibrils). A dense network of elastin fibres and microfibrils were seen in the superficial layer. Little elastin was found in the middle zone matrix, but staining was seen in cell nucei. Microfibril staining was primarily pericellular and within the inter-territorial matrix. The conclusion was the elastic network, in the superficial zone, may re-enforce the cartilage network during loading and help in recovery post loading.

ENOS null osteoblasts produce nitric oxide in response to fluid flow but do not translocate ß-catenin. **C Huesa** (Aberdeen, UK). Huesa (**P071**) described how endothelial nitric oxide synthase enzyme (eNOS) is activated in endothelial cells in response to mechanical stimuli, resulting in NO production. Mice lacking eNOS have a mild/transient low bone mass phenotype due to defects in osteoblast function and differentiation. To explore this osteoblasts from wild type and eNOS null mice were subjected to pulsatile fluid flow (PFF) and NO production measured in real time and ß-catenin localisation detected by immunofluorescence. Wild type cells had a constant increase in NO that was significantly reduced in eNOS null cells. Both cell populations demonstrated increased NO production shortly after the start of PFF, and production could be blocked with general and inducible NOS inhibitors. ß-catenin only translocated to the nucleus in wild type cells. Huesa concluded eNOs is not solely responsible for NO production following PFF, and that low basal levels of NO in eNOS null osteoblasts maybe responsible for the lack of ß-catenin translocation to the nucleus.

Anti-angiogenic properties of proteoglycan in cartilage explant cultures. **JJ Bara** (Owswestry, UK). Bara (**P076)** began by stating that vascularisation of osteoarthritic cartilage is associated with proteoglycan loss, and that aggrecan can inhibit the adhesion and migration of monolayer cultured

endothelial cells. Bara explored the relationship between cartilage proteoglycan loss and endothelial cell invasion. Bovine cartilage explants were enzymatically digested then seeded with fluorescently labelled endothelial cells. Proteoglycan release was measured on parallel cultured explants, and endothelial cell adherence to explants measured 4 hours and 7 days post seeding. Proteoglycan loss directly correlated with significant increase in endothelial cell adhesion. The endothelial cells adhered to the surface and deeper/calcified zones of the explants, but no proliferation or migration was observed during the 7 days of culturing. Bara concluded that an inverse relationship exists between cartilage proteoglycan content and susceptibility to endothelial cell adherence. The results also support the theory that proteoglycans are anti-angiogenic.
Increased fat mass and reduced serum osteocalcin in individuals with high bone mass: Possible cross-talk between fat and bone metabolism. **CL Gregson** (Bristol, UK). Gregson explained (**P130**) that mice models suggest there is cross-talk between fat and bone metabolism, which has been liked to osteocalcin. To determine if this cross-talk exists in humans 248 individuals with known high bone mass (HBM) were identified. Controls consisted of unaffected first degree relatives and spouses. Serum osteocalcin and P1NP (measurement of bone turnover) levels were measured and DXA scans used to evaluate bone mineral density. Initial results indicate that HBM individuals have increased total body fat mass and body bone mineral content, and decreased osteocalcin levels. The levels of P1NP and total body lean mass were similar between HBM cases and controls. Gregson concluded the results are consistent with cross-talk between fat and bone metabolism, which could involve osteocalcin.

Evaluation of bone marrow mesenchymal stem cell therapy for tendon regeneration in a large animal model. **NJ Young** (North Mymms, UK). Young (**P023**) described how tendinopathies are common in human and equine athletes, with healing resulting in scar tissue, reduced performance and increased re-injury rates. Mesenchymal stem cells (MSCs) were implanted into injured equine superficial digital flexor tendons (SDFT) and harvested 6 months post implantation to investigate if (MSCs) could regenerate tendon. Tendon matrix, cellularity, vascularity and composition were analysed. Results indicated MSC treated tendons had decreased cellularity, and tissue linked fluorescence, DNA and water were similar to that of uninjured tendon. MSCs treated sites had increased collagen and lower glycosaminoglycan compared to uninjured tendon. Young concluded that MSC transplantation results in tissue which closely resembles normal tendon matrix.

Calcium-induced secretion of DKK-1 in osteoblastic cells. **HS McCarthy** (Gobowen , UK). Dickkopf-1 (DKK-1) is a secreted inhibitor of Wnt signalling produced by many cell types including osteoblasts. To explore DKK-1 production McCarthy (**P053)** cultured osteoblastic cell line MG63 and measured production by ELISA and via qRT-PCR. Results showed calcium could increase DKK-1 at the protein level. Signalling inhibitors were tested, including ERK, Pi3K and JNK inhibitors but had no effect on calcium induced DKK-1. Calcium channel inhibitors and a metabotropic glutamate inhibitor also had no effect. There was no significant change in DDK-1 mRNA expression in response to calcium, but a significant increase in Wnt7b and a significant decrease in Wnt11 were seen. DKK-1 was significantly lower in lysates of calcium stimulated cells than non stimulated cells. McCarthy concluded calcium effects DKK-1 secretion rather than production.

The PPAR agonist Rosiglitazone reverses the persistent fibrotic phenotype of scleroderma fibroblasts. **X Shi-wen** (London, UK). Data comparing the response of control and sclerderma (SSc) fibroblasts to treatment with the peroxisome proliferator-activated receptor (PPAR) gamma agonist, rosiglitazone was presented (**P029)**. PPAR gamma regulates cell differentiation and its agonists were hypothesised to suppress the ‘persisitent fibrotic phenotype of SSc fibroblasts’. SSc fibroblasts showed increased alpha-smooth muscle actin, collagen I and CTGF expression and decreased PPAR expression. This phenotype was reversed in rosiglitazone treated cells.

Moreover, treatment decreased SSc migration *in vitro* and increased their ability to generate contractile forces.

The inhibitory role of suppressor of cytokine signalling-2 on STAT signalling in the growth plate. **C Pass** (Edinburgh, UK). The regulatory role of SOCS2 (suppressor of cytokine signalling2) in the growth plate was described (**P008)**. Pass compared signalling responses between costochondral cells from wild-type and SOCS2 knockout mice following treatment with GH, IGF-1, IL-1beta and TNFalpha. GH, IL-1beta and TNFalpha treatment increased STAT phosphorylation in control and $SOCS2^{-/-}$ cells, though STAT3 phosphorylation was higher in $SOCS2^{-/-}$ cells compared to control. GH-induced STAT activation in control cells lasted up to 60mins, whereas no subsequent decrease was seen in the $SOCS2^{-/-}$ cells for the time points studied. SOCS2 inhibited GH, IL-1 and TNF signalling through STAT activation in the growth plate, which has implications for chronic paediatric inflammatory conditions.

Lovastatin upregulates chondrocyte marker expression in tenocytes. **HR Cornell** (Oxford, UK). Cornell presented data (**P061)** that investigated the effects of statin treatment on primary tenocytes within the context of chondroplasia. Lovastatin treatment dose-dependently increased the chondrogenic transcription factors sox5, -6, and -9 mRNA expression and the induced the expression of type II collagen. Modulation of this pathway included regulation of the actin cytoskeleton and was likely to alter the tenocyte phenotype.

Mild hyperthermia promotes osteoclastogenesis whilst retarding osteoblast differentiation and bone formation. **JJ Patel** (London, UK). Patel described (**P055)** an interesting experiment investigating the effects of mild hypothermia on primary osteoblast and osteoclasts. Osteoblasts formed 75% less new bone structures *in vitro* at 35.5°C compared to 37°C. Osteoclast cell numbers and pit formation scores were increased for the lower temperature of 35.5°C compared to 37°C. This may explain the incidence of increased bone resorption and decreased bone formation (osteopenia/osteoporosis) in the elderly who often have lowered body core temperatures.

In situ ultrastructural imaging of native extracellular matrix macromolecules. **HK Graham** (Manchester, UK). Graham described (**P079**) the advantages and applications of their atomic force microscopy technique to examine various tissues. Sample processing, fixing and staining can likely affect the tissues under study and this method aims to minimise these potentially damaging processes. This technique could resolve images to nm-scale. Several tissue types were demonstrated and these included samples in or out of physiological buffers. The proposed their technique be useful for studying native protein and extracellular matrices, before and after treatments.

A role for TRPV1 and K+ channels in the regulation of osteoblast transdifferentiation to adipocytes. **NC Henney** (Cardiff, UK). Henney (**P041)** hypothesised that TRVP1 and BKCa ion channels regulate the transdifferentiation of osteoblasts into adipocytes. They exposed mouse 7F2 cells to TRVP1 or K+ channel ligands. Cells were stained for lipid and underwent RT-PCR for TRVP1 and BKC channels. TRVP1 agonists decreased lipid in the adipocyte-induced cells, whereas antagonists had no effect. K+ channel blockers increased lipid levels in the adipocyte-induced cells. TRVP1 and BKC mRNA was detected but no changes compared to control were reported.

Joint Symposium: Dynamic relationships with the extracellular matrix: lessons from dysplasias.

Chaired by Stefan Mundlos and Bjorn Olsen, Adam Engler (San Diego, USA) gave the first talk of the session entitled 'Intrinsic matrix properties direct stem cell fate: Insights into anomalous bone formation' (**IS21)**. He discussed the importance of using correct intrinsic cues to specify

differentiation to a phenotype. He opened his talk by reminding us that cell therapies with stem cells are only marginally functional at present. Stem cell morphogenesis is directed by substrate elasticity. With this in mind he tested nerve, muscle, and bone-like matrices in addition to commercial hydrogel-type scaffolds. For example, through placing MSCs on different hydrogel matrices they found soft matrices that mimic the brain cause cells to move into a neurogenic lineage, and hard matrices that mimic collagenous bone cause the cells to move into a osteogenic lineage. Engler explored this further by injecting MSCs cells into fibrotic infracts caused by cardiomyoplasty surgery. Cardiomyoplasty is a procedure which involves taking healthy muscle from one part of the body and wrapping it around a failing heart to provide support. They found that the cells injected into the infract expressed bone-like properties rather than muscle, and that differentiation appeared to be dependent on myosin-II. It seems that their cell therapy was indeed working but this went unrecognised since they were looking for the wrong outcome. Naive MSC given to ischaemic muscle scar tissue developed into osteoblasts rather than muscle. This was due to the abnormally stiff matrix and stiff matrices are osteogenic in nature. In addition to the stiffness, matrix composition should be considered since this could also determine cell differentiation, for example, a high collagen II component for chondrogenicity. Furthermore, truncated ECM molecules could prevent key developmental events such as gastrulation. Engler concluded that these findings suggest a broad application of intrinsic matrix properties as differentiation regulators for stem cells, and that elasticity and ligand coating need to be considered when selected implants for cardiomyoplasty surgery.

The second talk of the session (**IS22)**, entitled 'Fibrous displasia – Models of disease, models of therapy' was delivered by **Paolo Bianco** (Rome, Italy). He described fibrous dysplasia (FD) as a disease that causes growths and lesions in one or more bones, leading to bone weakness and scar formation. The skeletal lesions are characterized by the replacement of healthy bone and bone marrow with abnormal bone containing fibrotic marrow devoid of haematopoietic elements. There is a complicated relationship between the genotype and phenotype of the disease and thus only partial understanding about pathogenesis and skeletal expression. There is no cure and no good treatments beyond palliation. The disease results from sporadic, post zygotic mutations in Gs-alpha (*GNAS*), which encodes for an activating G-protein $G_s\alpha$. The two most common mutations are replacement of arginine with a cysteine or histidine. These mutations lead to a loss in intrinsic GTPase activity of $G_s\alpha$, causing activation of adenlyly cyclase and increased cAMP production. The mutations lead to hyperpigmentation and hyperfunctioning endocrinopathies in sufferers. The clinical course is quite variable and depends on extent of disease and accompanying endocrinopathies. Bianco explained that the range of phenotypes and disease severities is caused by the mutations being within pluripotent embryonic stem cells and the subsequent migration and misfunction of their differentiated progeny. The mutation itself occurs prior to gastrulation. He stated that clinical and pathological studies are crucial for understanding the disease and have already revealed that FGF-23 plays a role in the disease. To understand the disease further Bianco described how two mouse models of the disease were generated, the first mouse only expressed the causative mutation in the osteoblasts and the second model had ubiquitous expression of the mutation. These two mouse models were compared to a human stem cell line which also expressed the causative mutation in the same vector as the mouse model. These have revealed novel and potentially important aspects of the disease such as adaptive responses within cells to cope with the effects of the mutation and specifically targeted vs. ubiquitinated transgenes have an effect on specific tissue changes and clinical bearing.

The final talk of the meeting was given by **Ray Boot-Handford** (Manchester, UK) and entitled 'ER (endoplasmic reticulum) stress as a pathogenic factor in chondroplasia'. He began the talk (**IS23)** by describing the complicated machinery within the endoplasmic reticulum (ER) that promotes protein folding and copes with misfolding. A series of stress sensors maintain folding homeostasis within the

ER by promoting a down regulation of protein synthesis, an increase in protein folding chaperones and activation of proteosomal and autophagic protein breakdown, in response to increased levels of misfolded protein. This process is known as unfolded protein response (UPR) and has been shown to be activated in chondrocytes in response to expression of mutated extracellular matrix genes. He stated that ER stress plays a key role in metaphyseal chondrodysplasia type schmid (MCDS), a dwarfism caused by an autosomal dominant mutation in the collagen type X gene. Collagen type X is mainly restricted to the hypertrophic chondrocytes of the growth plate and the MCDS phenotype can be recapitulated through targeting ER stress in hypertrophic chondrocytes in the mouse. The MCDS mutation in mice clusters at the C-terminal of type X collagen. X-ray analysis of these mice at 10 weeks showed shortened bones and immunohistological analysis showed an expansion of the hypertrophic zone with an increase in collagen X within the cells, compared with wild type. The hypertrophic zone in the mouse model is enlarged due to chondrocytes activating the unfolded protein response (UPR) pathway in response to ER stress, which resulted in reduced chondrocyte proliferation. It was concluded that the pathology was a direct response to an increase in the unfolded protein response. The take-home message was that pathologies are not always the result of mutated proteins *outside* of the cell and that ER stress should be taken into account and worked with, in terms of therapy.