

Conference report for Spring 2008 BSMB meeting (York)

The Spring meeting of the British Society for Matrix Biology AGM, 2008, was held on April 7th and 8th in the mediaeval city of York. As a city rich in historical findings giving forth to an abundance of early blooms, York provided the perfect backdrop for a comprehensive meeting focusing on past, present and future insights into understanding and treating osteoarthritis (OA). The 122 delegates (represented by 52 full members, 23 student members and 47 non-members) were treated to an international and high quality series of 13 plenary and 18 short talks, expertly organised by Philippa Parsons. The meeting was very kindly sponsored by Smith & Nephew, The Company of Biologists, Promocell, R&D Systems, Applied Biosystems, Lonza, BD Biosciences, TebuBio, Cambridge Research Biochemicals and PAA.

The molecular and mechanical aspects of osteoarthritis were covered over 6 sessions focused on the When? Why? and How? of this disease, with further discussions on disease models and treatments. A need for consideration of both local and systemic disease responses was highlighted, as were the similarities between OA and ageing, which could be exploited to understand the mechanisms involved in both processes. The audience heard about destructive and chondroprotective processes in cartilage; detailed knowledge of both is essential in tackling this disease pertinent to an ageing population. The sessions were not limited to research findings and future perspectives, but also contained important take-home messages for both young (apply for any funding you can get your hands on, collaborate and exercise) and old(er) alike (support your young blooms, collaborate or die, and exercise), and perhaps most importantly of all, tips on how to persuade guinea pigs to run.

An upcoming and talented researcher, Dr. Emma Blain (Cardiff University), was the recipient of the Young Investigator Award, and gave an overview of her investigations into the key role played by the chondrocyte cytoskeleton in cartilage metabolism (below). Four young researchers, Kirsty Culley (University of East Anglia, Norwich), Eleanor

Jones (University of East Anglia, Norwich), Andrew Marson (Manchester University) and Natasha Baker (University of East Anglia, Norwich) were supported by conference presenter bursaries from the BSMB, and the meeting was reported on by the BSMB reporter bursary recipients, Kate Silverthorne, Rachel Oldershaw (both University of Manchester), and Lorna Fiedler (Cardiff University). The International Journal of Experimental Pathology (IJEP) generously sponsored three poster prize awards of £150, which went to Natasha Baker (University of East Anglia, Norwich), Athanosios Didangelos (Kennedy Institute, London) and Ngee Han Lim (Kennedy Institute, London). Of course, the meeting also could not have taken place without the support of our generous sponsors: Smith and Nephew, The Company of Biologists, IJEP, PromoCell, Applied Biosystems, Lonza, R and D Systems and BD (Becton, Dickinson and Company).

It should also be noted that the aforementioned bursaries would not have been possible without the BSMB membership fees. Membership therefore provides not only perks such as copies of the aptly named ‘Connective Issues’, reduced meeting fees and the opportunity to have a say in the direction of the society, but you can also be assured that your hard-earned cash is supporting and securing the future of matrix biology for years to come.

Session 1: “When?”

The first speaker of the meeting was Dr Virginia Kraus, of Duke University Medical Center, who gave a plenary talk about “Biomarkers and biomarker patterns: new and emerging trends to distinguish osteoarthritis disease subsets and progression.” Dr Kraus highlighted the current shortcomings in the diagnosis of OA, often relying on end-stage radiographic detection as there is no validated method for reliably identifying disease progressors. To address this, 462 female participants enrolled on the Genetics of Generalized Osteoarthritis (GOGO) program were analysed. These individuals underwent radiographic evaluation of hands, spine, hips and knees, and graded with regard to osteophytes (OST) and joint space narrowing (JSN). These were compared to 3

osteoarthritis biomarkers: serum hyaluronan (sHA), serum cartilage oligomeric matrix protein (sCOMP) and urinary C-telopeptide of type II collagen (uCTXII). It was observed that correlation of each biomarker to radiographic data altered between tissues and disease indicators. For example, sHA and uCTXII correlated with JSN, whereas sCOMP inversely correlated with JSN, at least in proximal interphalangeal OA. uCTXII correlated with knee and hip OA, in contrast to COMP, which correlated with spine OA. It was notable that concentrations of biomarkers were not proportional to the size of joint affected. In combination with age and Body Mass Index as predictors for OA, sCOMP and sHA increased predictability of disease, although hip OA was difficult to predict, possibly due to variations in radiographic imaging. It was hypothesised that more standardised radiographic methods would lead to greater correlation with biomarkers. As different biomarkers correlated to different diseased joints, it would be necessary to consider the whole body when phenotyping the total disease burden of OA.

The second speaker of the session was Professor Alan Boyde from the Dental Institute, London, who spoke on “X-ray microtomography and scanning electron microscopy in study of loss of articular calcified cartilage and subchondral bone in human femoral head”. These methods, in combination with confocal microscopy, enable visualisation of deep structures within the tissue without damage incurred by physical sectioning. Using these techniques, Professor Boyde demonstrated the progression of the calcified cartilage front from underlying bone towards the hyaline cartilage in the osteoarthritic femoral head. He also revealed areas of trabecular bone microfracture, leading to densely woven bone within the bone marrow space. Areas were clearly evident where the cartilage had become detached from the bone, increasing with age and osteoarthritis. Furthermore, holes from the subchondral bone through to the mineral front were caused by osteoclast-driven digestion. However, why these osteoclasts, which are also capable of digesting cartilage, halt their progress 50 µm short of the cartilage surface remains unknown.

Natasha Baker, from the University of East Anglia, spoke next about Wnt1 inducible secreted protein 3 (WISP-3) in osteoarthritic cartilage and its role in the canonical Wnt signalling pathway. Mutations in this gene have been identified in Progressive

Pseudorheumatoid Dysplasia (PPRD). Quantitative PCR and immunohistochemistry revealed WISP-3 is upregulated in osteoarthritic cartilage compared to normal and is mainly localised to the pericellular matrix. Stable overexpression of WISP-3 in the chondrocytic cell line C-38/I2 resulted in downregulation of MMPs 2, 14, 15, TIMPs 2 and 3, ADAMTS4 and 5 but substantial upregulation of MMP10. As it has been previously reported that WISP3 inhibits canonical Wnt signalling the effect of Lithium chloride (LiCl) on WISP3-overexpressing cells was investigated, as LiCl activates β -catenin in the canonical Wnt signalling pathway. They found that LiCl partially reverses WISP3-induced downregulation of ADAMTS5 but enhanced MMP10 expression, suggesting a complex mechanism for control of MMP10. Addition of IL1 α /Oncostatin M (known to regulate MMP10) repressed the MMP10 upregulation seen in WISP3-overexpressing cells. In the disease PPRD, It was hypothesised that the mutated WISP3 cannot inhibit Wnt signalling effectively. The upregulation in OA cartilage may be part of a reparative response and it was speculated that commercial inhibitors of Wnt signalling may have a similar effect to WISP3.

The next speaker was Elizabeth Barr from the University of Liverpool, talking about biomarker alteration in cartilage and the subchondral bone of horses with Palmar Osteochondral Disease (POD). This condition is a naturally occurring OA, resulting from trauma, in which the subchondral bone becomes fragmented followed by cartilage collapse. As such it is a valuable model to investigate the role of the subchondral bone in OA. Gene expression in the subchondral bone and cartilage from horses with this condition was analysed by quantitative PCR. In both tissues MMP3 was upregulated with POD whereas TIMP-3 and biglycan were downregulated, with a significant correlation between these genes. MMP1, MMP13, collagen types I, II, X and IL1 β levels remained unchanged. As MMP3 cleaves both type II collagen and aggrecan, this suggested a net collagen and proteoglycan loss having a role in the pathogenesis of POD. The lack of change of the other genes was hypothesised to be a feature of the group of animals used in this study, as previous work revealed that tissue with a much higher Mankin score than those used here (at least 9) exhibited an upregulation of MMP13. This

study suggested a level of synchrony in gene expression between the cartilage and subchondral bone, highlighting the role of the latter in the development of OA.

The last speaker of the session was Professor Stefan Lohmander, from Lund University in Sweden, with the engaging title “When I'm sixty-four...: Young patients with old knees - exploring early-stage OA with human models and biomarkers.” He highlighted the multi-factorial nature of OA resulting from a mixture of genetic and environmental components. OA is the major cause of disability in the adult population including the working, younger population, although age is a risk factor. Other factors include obesity, lifestyle, genetics, injury, overload, and instability of the joint. As a result, knee injuries are common and following anterior cruciate ligament injuries, 10 years later 50% of patients have developed OA, that percentage increasing over time. Furthermore, surgical intervention such as meniscal repair, autografts and allografts seem to offer no long term benefit. Following surgery, the patient may feel improvement for 2 years, but the joint deteriorates as OA develops. Interestingly, an increased prevalence is also observed in the patient's other uninjured knee. This damage is viewed as accelerated joint ageing, with the OA being at a similar grade to individuals without injury but 25-30 years older; patients with a heredity disposition to OA also exhibit this accelerated joint ageing. Subsequent to meniscal tear, the likelihood of developing OA depends on a number of factors, such as a body mass index (BMI) over 30 (4 times more likely), being female (3 times more likely) and having hand OA, a more hereditary condition (3 times more likely). As discussed by Virginia Kraus at the beginning of the session, detection of OA is done radiographically, by which time the disease is in the final stages; biomarkers are receiving significant interest as a means of earlier diagnosis of OA. Professor Lohmander's group is mapping different cytokines in synovial fluid following injury and analysing aggrecan and associated fragments. They have found that the different neoepitopes generated from aggrecan degradation (from both aggrecanase and non-aggrecanase activity) are observed immediately after injury, but subsist after several years. This complex combination of aggrecanase- and non-aggrecanase- derived fragments appear to differ between types of OA. For example, two major fragments result from cleavage in the CS1 and CS2 domains. The ratio of these species changes

between different joint diseases and analysis of the types of aggrecan fragments may provide a means of more accurate diagnosis, as well as indicating the activity of the joint at the molecular level.

Session 2: “Why?”

The session opened with a plenary lecture from Professor Donald Salter (University of Edinburgh) who presented “Cartilage and chondrocyte mechanotransduction in osteoarthritis”. Professor Salter introduced the concept that cartilage homeostasis was affected by mechanical stimuli and that tissue thickness was proportional to load. Dynamic movement under physiological parameters provides an anabolic stimulus which increases synthesis of TIMPs, collagen, proteoglycans and sGAG. Conversely, abnormal mechanical stress including obesity, injury and muscle weakness creates a pathological stress which has a catabolic effect leading to the production of prostaglandins, nitric oxide and proteases. Investigating the differences between physiological and pathological loading and the subsequent biomechanical effects will provide understanding as to how chondrocytes produce and maintain cartilage matrix within a healthy joint. Mechanical loading is applied to the cartilage and causes chondrocyte deformation leading to multiple signalling events. Experimentally, mechanical loading can be measured in terms of dynamic loading defined by frequency and duration, static loading, compressive loading and shear forces with model systems including *in vivo* studies, *in vitro* studies, explant cultures, 2D cultures and 3D cultures. Experiments carried out on monolayer, explant and 3D agarose chondrocyte cultures have shown that dynamic loading under physiological parameters activates Ca^{2+} , PLC, MAP kinase, cAMP and CAM kinase signalling pathways leading to an anabolic increase in sGAG and aggrecan production. The transcription factors c-fos and c-jun are seen to be upregulated within 1 hour of mechanical loading but SOX9 gene expression is not significantly upregulated above control levels until after 4 hours. The response of chondrocytes to mechanical stimuli introduces the concept of “mechanocoupling” whereby forces acting on the macroscopic tissue are translated into local action within the pericellular environment of the cell, in particular the activation of matrix-bound growth factors and the stimulation of

mechanoceptors on the surface of the chondrocyte. One example is the enzymatic release of FGF-2 from perlecan, causing the release of soluble ligand to act on the cell receptor. Mechanical stimulation of human chondrocytes at 0.33 Hz causes hyperpolarisation of the cell membrane within 20 minutes as an effect of potassium efflux from the cell. This response has been shown to be modulated by cell-ECM interactions and is maximal with fibronectin substrate, suggesting that the integrin $\alpha 5\beta 1$ acts as a mechanoreceptor in human chondrocytes. Indeed, integrin $\alpha 5\beta 1$ inhibition by function blocking antibodies and RGD-containing oligopeptides results in a decrease in membrane hyperpolarisation following mechanical stimulation and reduced aggrecan mRNA expression. Recent data from Professor Salter's laboratory has also shown that the integrin-associated molecule CD47 has a membrane hyperpolarisation response to 0.33 Hz stimuli which is inhibited by a function blocking antibody. Similarly, mechanical induction of PKC and its regulatory protein RACK1 causes membrane hyperpolarisation. Mechanical forces exerted on human chondrocytes have been shown to increase secretion of soluble paracrine and autocrine factors particularly IL4 and substance P. Substance P can modulate cell adhesion to fibronectin through upregulation of $\alpha 5$ integrin expression, suggesting that a positive feedback loop is established during chondrocyte mechanotransduction. Professor Salter proceeded to talk about how abnormal loading to articular cartilage leads to the onset and progression of OA. Whereas dynamic loading to intact cartilage explants is anabolic, static compression has an inhibitory effect on collagen type II and proteoglycan production. Acute and chronic trauma to the tissue causes activation and expression of pro-catabolic mediators such as reactive oxygen species, nitric oxide, COX-2 and prostaglandins, resulting in apoptosis of chondrocytes via caspases 3 and 9 and loss of proteoglycan. Disruption of matrix integrity alters the biomechanical properties of the tissue and hence modulates the mechanical stimuli received by the chondrocyte leading to catabolic remodeling of the matrix. Recent data has revealed that different loading regimes can affect the dynamics and amounts of MAPK signalling pathway components within chondrocytes. ERK1/2 increases modestly in response to dynamic shear but is more strongly upregulated in response to continuous static compression. In contrast, p38 was transiently upregulated by static compression but was only activated much later under dynamic shear conditions. These results clearly

demonstrate how the mechanical environment alters the mechano-responsiveness of chondrocytes. Additional studies have shown that bovine articular chondrocytes fail to respond to anabolic stimuli post-injury. Similarly, in contrast to normal chondrocytes, OA chondrocytes exhibit no change in sGAG production following mechanical stimulation under physiological parameters giving insights into how OA progresses within the joint through failure in replacing lost matrix. In concluding his presentation, Professor Salter emphasised the importance of understanding the mechanotransduction mechanisms which mediate chondrocyte metabolism in order to develop appropriate drug regimes in treating OA and optimising protocols for tissue engineering strategies.

The second talk of the session was from Blandine Poulet (Royal Veterinary College) who discussed “*In vivo* knee joint loading of Str/ort mice: distinguishing between load-induced and naturally occurring lesions”. Miss Poulet stated that the combinatorial effects of genetics and *in vivo* joint loading are known to cause OA though few models are available for studying the mechanisms by which disease onset and progression is initiated. Previous data had shown that *in vivo* joint loading on normal joints of C57BL/6 mice lead to the formation of lesions and Miss Poulet questioned if this loading could affect the development of lesions in the joints of OA-prone Str/ort mice. The right knee joints of 22 week old male Str/ort mice were subjected to a regime of 40 cycles at a magnitude of 9 N and a frequency of 0.1 Hz, 3 times per week over a period of 4 weeks; mice were sacrificed 2 days after the final session. Sectioned knee joints were stained with toluidine blue and graded numerically with 0 representing normal cartilage and 6 being severe (greater than 80%) loss of the articular cartilage surface. Miss Poulet showed that natural lesions were apparent in the medial and lateral tibial compartments on the non-loaded contra-lateral control joints taken from the left knee of the mouse. Loading was shown to increase the mean grade observed but had no significant effect on the maximum grade. In addition, mechanical loading was only seen to increase both the maximum and mean grade of lesions in the lateral and medial femoral compartments, the tibial compartments remained unaffected by loading. From this, Miss Poulet concluded that mechanical loading induces new lesion formation on the femur of Str/ort mice, as observed with C57/BL6 mice, but does not affect the severity of spontaneously occurring

lesions in the tibia. She concluded that the *in vivo* joint loading regime presents a novel non-invasive model for the induction of OA. This model could be applied to different genetic backgrounds as well as genetically-modified mice to investigate further the role of genetics and mechanics in the development of OA.

Ruth Webster (AstraZeneca) presented the next presentation entitled “Osteoporosis alters the underlying subchondral bone integrity in an experimental model of osteoarthritis in rats”. Miss Webster began by introducing background information from clinical cases and rabbit models showing that localised osteoporosis can weaken and alter the architecture of subchondral bone leading to increased severity of the articular surface. The aim of the study was to investigate the effects of osteoporosis on the progression of OA using a rodent model that could produce rapid and reproducible results for drug discovery purposes. Osteoporosis was induced by castration or in combination with bilateral adrenalectomy. OA was induced 5 weeks post-osteoporosis induction by transection of the medial meniscus (MMT) and animals were sacrificed 5 weeks later. Miss Webster’s results showed that surgical castration and/or adrenalectomy, causing a reduction in testosterone levels, significantly decreased femoral length compared to non-operated control rats. In addition, dual x-ray absorptiometry (DXA) showed reduced bone mass density and therefore it was concluded that operated animals had developed osteoporosis. Analysis of joints taken from MMT on non-osteoporosis induced animals showed osteophyte formation and loss of proteoglycan when compared to contra-lateral joints taken from the same animal and presented pathology consistent with the onset of OA. When studying the combinatorial effects of osteoporosis and OA Miss Webster found that induction of osteoporosis by castration alone had no additive effect on the pathology of OA following MMT but cartilage deposits were observed in the subchondral bone. In contrast, joints taken from animals that had undergone adrenalectomy, castration and MMT showed less proteoglycan loss, minimal matrix loss, less fibrillation and smaller lesion size suggestive of a protective effect on the integrity of the articular surface. The joints taken from these animals also presented with increased frequency of cartilage deposits in the subchondral bone. Miss Webster concluded that that the adrenals had a greater contribution to bone loss than castration alone and that turnover in bone

architecture was influential in altering articular cartilage matrix integrity. Furthermore, this rodent model could be applied to the testing of novel drug regimes.

The next presentation of the session titled “A chondroprotective role of matrix bound FGF-2” was given by Dr Tonia Vincent (Kennedy Institute of Rheumatology). Previous work by Dr Vincent has shown that FGF-2 co-localises with perlecan within the pericellular matrix surrounding the chondrocyte and is released when articular cartilage is cut or mechanically loaded leading to the subsequent activation of the MAP kinase ERK1/2. The aim of the study was to investigate the *in vivo* effects of FGF-2 on articular cartilage using FGF-2 knockout mice and a destabilisation of the medial meniscus (DMM) model which results in the spontaneous onset of OA. Fgf-2^{-/-} mice were viable, fertile and morphologically indistinguishable from wild-type mice. Furthermore, histochemical analysis of the cartilage revealed no differences in tissue thickness and proteoglycan staining. OA was induced by DMM at 12 weeks of age and the cartilage analysed 4 weeks post-surgery. Histological analysis of joints taken from wild-type DMM mice showed significant loss of cartilage when compared to sham operated control mice, indicative of the onset and progression of OA. The loss of cartilage from the joints of DMM-operated Fgf-2^{-/-} mice was significantly more severe than that seen in the wild-type, with complete erosion to the subchondral bone. Dr Vincent presented gene expression data showing that ADAMTS5 was significantly upregulated following DMM in Fgf-2^{-/-} mice compared to the wild-type control and this correlated with additional data from their laboratory showing that FGF-2 could suppress IL-1 mediated aggrecanase activity in human articular cartilage explants. From these data Dr Vincent concluded that FGF-2 was an endogenous chondroprotective factor that acted by suppressing the activity of ADAMTS5.

The session was closed by a plenary presentation from Dr John Loughlin (University of Newcastle) entitled “The role of genetic susceptibility in osteoarthritis”. Dr Loughlin began by introducing the concept of twin studies to identify the effects that genes have on the risk of developing OA. Genetic diseases such as cystic fibrosis have a concordance rate of 100% in monozygotic twins and 25% in dizygotic twins. In comparison, the

concordance rate of OA is 55% in monozygotic twins and 20% in dizygotic twins indicating that the disease is not influenced by genetics alone. Furthermore, the heritability of OA is dependent on the site of the joint, with concordance rates varying from 40-60%. Dr Loughlin went on to explain that OA is a multifactorial condition being influenced by the environment as well as the heritability of many genes, some of which have more influence over the development and progression of the disease than others. Genome-wide linkage scans and candidate gene-based associated studies are being used to identify alleles which predispose OA. Of particular interest is a polymorphism in the 5'-UTR of GDF5 (rs143383) which associates strongly with hip OA in Asian populations with a T-allelic frequency of 84% in OA cases compared to 74% in the controls. GDF5 is a member of the TGF β superfamily and is involved in both joint development and maintenance of the adult tissue and as such would be a prime candidate for involvement in OA development. A gene-based associated study was carried out on a European cohort with a larger number of cases than the Asian study and revealed that the GDF5 polymorphism was associated with 64.4% of OA cases compared 62% in the control. Inheritance of the T-allele was shown to increase the risk of developing OA, but this effect was weaker in Europeans than in Asians. Experiments to determine the functional differences between the T and C allele were carried out using promoter-luciferase assays in a chondrogenic cell line and revealed a decrease in transcript expression from the T-allele when compared to the C-allele control. *In vivo* analyses of OA cartilage obtained from patients who were heterozygous for the T-allele showed a decrease in transcript expression compared to the C-allele. Further to this, less T-allele expression and subsequent protein production was observed in multiple tissues of the joint including ligament, synovium, fat pad and meniscus. From this data Dr Loughlin concluded that there was a global association of regulatory polymorphisms with GDF5 in OA. As cartilage was not the only tissue to be affected by reduction of T-allele expression, this suggests that OA is a whole organ disease. The second part of Dr Loughlin's presentation introduced a collaborative project termed arthritis research campaign osteoarthritis genetics (*arc*OGEN) involving nine academic centres around the UK (http://www.arc-research.org.uk/med_director/arcogen.asp). At £2.2 million *arc*OGEN is the single largest grant awarded by the ARC and will carry out a genome-wide association scan

using 500,000 SNPs on 8,000 OA cases obtained from hip and knee joint replacement surgeries of both male and female patients. Allelic gene frequencies within OA cases will be compared to 6,000 control cases using an Illumina 610 quad array. Presently, all 6,000 control cases and the first 4,000 OA cases have been collected and it is anticipated that the collection and genotyping of the second cohort of 4,000 OA cases will be completed by summer 2009. Dr Loughlin predicted that the fully analysed data will be available as an open resource by the end of 2009 and will present the most comprehensive analysis of genetic influence on the development and progression of OA.

The scientific programme was briefly interrupted by Professor Alan Silman (University of Manchester, ARC Medical Director) who discussed the opportunities for research funding from the ARC and in particular fellowship applications. Professor Silman showed that in recent years the ARC has funded up to 40% of fellowship applications demonstrating financial commitment to the development and progression of careers with the field of musculoskeletal research. Future applications were encouraged from young researchers who had 3-6 years of post-doctoral experience and who had throughout this time proved active in the development of their own particular area of research through the publication of original research articles. Professor Silman also suggested that potential candidates could increase their publication record through writing review articles based around their particular area of expertise. It was also stated that principal investigators had a responsibility to provide exciting and engaging projects for young researchers so that they remained committed to developing a career in research.

The first day of the meeting concluded with the BSMB Young Investigator Award presented to Dr Emma Blain by Professor Bruce Caterson (Cardiff University), Professor John Couchman (University of Copenhagen) and Dr Graham Riley (University of East Anglia, Norwich). Dr Blain's presentation entitled "The role of the cytoskeleton in articular chondrocyte metabolism" encompassed early data from her PhD studies through to current investigations to give interesting insights into how mechanical stimulation coupled with the arrangement of chondrocyte cytoskeletal components controlled matrix homeostasis. Dr Blain began by introducing articular cartilage as an avascular,

alymphatic and aneural tissue which has an extensive extracellular matrix maintained in a metabolic balance of anabolism and catabolism. Whilst mechanical loading is integral to the regulation of matrix homeostasis, abnormal loading (outside physiological parameters) has been shown to cause catabolic imbalance within the tissue and is one risk factor in the multi-factorial disease condition of OA. Previous studies had shown that the expression of the MMPs 1, 2, 3, 7, 8 and 9 was increased in OA and Dr Blain was interested in investigating the mechanisms by which mechanical loading regulated specific gene expression leading to the upregulation of these degradative enzymes. Articular cartilage explants were subjected to cyclic compressive loading of 0.5 MPa, 1 Hz (equivalent to physiological loading) for 10, 30, 60 and 180 minutes and demonstrated significant increase in expression of activated MMPs 2 and 9 when compared to unloaded control explants taken from the same joint. Differential RNA display technology compared gene expression of loaded and unloaded control explants and identified a 20-fold increase in thymosin β_4 mRNA after 10 minutes of loading. Immunolocalisation studies identified thymosin β_4 protein to discrete foci around the chondrocyte nucleus after 60 minutes of loading, absent in the control cultures. Furthermore, addition of exogenous thymosin β_4 to chondrocyte cultures caused a significant upregulation of MMP9 synthesis and activation. Thymosin β_4 was described as a 5 kDa polar peptide which sequesters G-actin within the cell and prevents actin polymerisation. Notably, previous studies had shown that thymosin β_4 upregulated MMP expression and activation in endothelial cells and changes in cytoskeletal architecture in chondrocytes were associated with OA. These data led Dr Blain to investigate a mechanistic link between cytoskeletal organisation and the regulation of MMP activity controlling matrix metabolism. Actin microfilaments confer compressive resilience upon the chondrocyte, induce chondrogenesis and help to maintain the chondrocyte phenotype. Dr Blain showed that treatment of chondrocytes with 1 μ M cytochalasin D resulted in disassembly of actin microfilaments without any affect on the organisation of tubulin and vimentin networks. F-actin disruption inhibited collagen type II synthesis and led to significant degradation of collagen type II due to increased activity of MMPs 2 and 9. Cytochalasin D treatment had no effect on TIMP activity. Dr Blain then investigated the role of vimentin intermediate filaments in chondrocyte metabolism. The assembly and

disassembly of vimentin filament networks is highly dynamic and is regulated by phosphorylation events, suggesting a potential mechanism in mechanical signal transduction. Dr Blain showed that vimentin intermediate filaments appear more loosely packed in chondrocytes obtained from OA cases than those from normal cartilage. Chondrocytes were treated with 5 mM acrylamide over a period of 3 days which had no effect on the total amount of vimentin protein within the cells but resulted in the complete disruption of spatial organisation and collapse of the filament network around the chondrocytes' nuclei. Treatment of chondrocytes with acrylamide resulted in dose-dependent inhibition of *de novo* collagen type II synthesis concomitant with increased phosphorylation of ERK1/2. Subsequent inhibition of ERK1/2 phosphorylation with 20 μ M PD98059 was able to partially restore the chondrocyte phenotype. The third cytoskeletal component investigated was tubulin microtubules. The tubulin microtubule network plays essential roles in protein trafficking and secretion, organelle distribution within the cell, spindle formation during mitosis and has been shown to be disrupted in human OA. Treatment of chondrocytes with 1 μ M colchicine caused spatial disorganisation of tubulin microtubules and significantly inhibited the expression of β -tubulin mRNA and protein but had no effect on the actin and vimentin networks. Colchicine treatment was shown to significantly inhibit collagen type II and sGAG biosynthesis and also reduce expression of the potent collagen type II inducing growth factor, TGF β 1. When colchicine-treated chondrocytes were cultured with exogenous TGF β 1 collagen type II mRNA and protein synthesis was inhibited but later rescued when the tubulin microtubule networks were stabilised with 1 μ M taxol. Dr Blain summarised by emphasising the correlations between chondrocyte cytoskeletal turnover and tissue homeostasis with its subsequent implications on the development and progression of OA. Ongoing studies will elucidate further the signalling mechanisms involved in regulating these metabolic events with preliminary data suggesting that phosphorylation of SMADs and the MAP kinase members ERK1/2 and p38 are particularly relevant.

Session 3: “How?”

Professor Jeremy Saklatvala (Kennedy Institute, Imperial College, London) began the session with an overview of the relationship between mechanical and molecular factors in OA. He described the first reports on the metabolic activity of articular cartilage, which characterised age-related changes similar to those observed in OA; namely progressive loss of proteoglycan matrix components and chondrocytes from the cartilage surface. He also described how loss of matrix components leaves the tissue more susceptible to mechanical damage. On the molecular level, factors such as IL-1, IL-17 and TNF- α promote proteoglycan (aggrecan) loss, while IGF-I, FGF-2 and TGF- β act as chondroprotective agents. However, soluble factors alone cannot explain the entire pathophysiology of OA. Interestingly, scarification (multiple, vertical cuts) of the surface of articular cartilage mimics the phenotype of OA, indicating that mechanical insults (injury) may precede or at least contribute to tissue degeneration. Injury triggers signalling pathways including the MAP kinases JNK, p38 and ERK, which activate transcription factors such as NF- κ B and the JNK dependent ATF2 to upregulate proteins involved in the inflammatory response. Additionally, mechanical injury causes JNK activation some distance away from the source of damage. Further, after wounded tissue was allowed to 'rest', a second insult released FGF-2 and activated ERK (similar to the initial wound response), but not JNK. This pathway also was not activated by conditioned media from wounded cartilage, indicating that soluble factors may not be responsible. It was suggested that on mechanical injury, an inflammatory 'alarm' response is triggered, and the lack of a second response indicates tissue adaptation. Overall, it was suggested that on mechanical injury, cell-surface matrix receptors allow cells to sense and respond to matrix damage by activating inflammatory responses and proteases. This leads to further matrix degradation that leaves collagen fibres more susceptible to tearing by mechanical load, thus exacerbating the cellular response. The key question for further investigation is exactly HOW mechanical injury leads to the cell-mediated immune response.

The second speaker, Ngee Han Lim (Kennedy Institute), described three N-TIMP3 mutants which can discriminate between ADAMTSs and MMPs. Wild type TIMP3 is a natural MMP inhibitor with broad specificity for both MMP and ADAM family

members. Indeed, the difficulty in specifically targeting single family members for therapeutic purposes lies in the highly conserved structural features. Many synthetic inhibitors therefore interact with multiple members, resulting in undesirable side-effects. Three mutants of the N-terminal region of TIMP3 were described, with an additional one or two alanine residues at the N-terminus, or a mutation at Threonine-2 (which binds the S1 specificity pocket of metalloproteinases). These inhibitors were used to investigate the relative contribution of the aggrecanases ADAMTS4 and 5 and MMPs in OA. The mutants were able to inhibit ADAMTS but not MMP activity, and were successfully used to reduce degradation of the ADAMTS4 and 5 substrate aggrecan in cartilage explants, indicating that this process is MMP independent. However, collagen degradation was not sensitive to these mutants, suggesting that ADAMTSs are not involved. That collagen degradation was sensitive to inhibition by wild type TIMP3 confirms that this process is instead mediated by MMPs. Interestingly, a TIMP1 sensitive MMP was present in human but not in porcine cartilage, although it was also noted that this protein may become apparent in later stages of OA in the porcine model.

Dr Athanasios Didangelos (Kennedy Institute) gave a talk on the mechanisms by which the cholesterol transporter protein APOA1 induces aggrecan breakdown. He described the initial observation that synovial fluid induces activation of MAP kinase signalling pathways in cartilage explants, resulting in an immune response and enhanced protease activity, and that this soluble factor was a protein (since the activity was abrogated by heat or trypsin degradation). To identify this factor, synovial fluid was subjected to chromatographic separation and active fractions were isolated. These were purified further and a candidate protein, APOA1, was isolated. The involvement of this protein was confirmed by the observation that the activity of synovial fluid was abrogated by immunodepleting APOA1. Isolated APOA1 by itself was also sufficient to induce these effects, similar to commercially available recombinant APOA1. It was demonstrated that APOA1 induced aggrecan breakdown by upregulating MMP13 and ADAMTS4 and 5, although interestingly it could not activate classical inflammatory mediators such as COX2. APOA1 is synthesised in the liver and transported in the serum as a monomer, which in its trimeric form binds high-density lipoprotein (HDL) in tissue fluids. A

working hypothesis is that APOA1 can only gain access to damaged cartilage to elicit a degradative response, thus providing a possible mechanistic link between injury and cartilage degradation.

Elaine Garvican from the University of Liverpool described a study on the phenotype of chondrocytes isolated from equine Osteochondritis Dissecans cartilage. In this disease, growing cartilage fails to differentiate, leading to abnormal thickening. This abnormally retained, immature cartilage develops fissures, triggering an inflammatory response and eventually resulting in an osteoarthritic phenotype. The main question asked by this study was whether these cell-mediated changes (pericellular matrix loss, decreased chondrocyte viability) are a result of permanently altered behaviour, or whether this could be reversed and the cells re-differentiated to a normal chondrogenic phenotype. Analysis of isolated cells at the point of OA indicated a switch to a degradative phenotype, evidenced by changes in MMP and MMP inhibitor protein levels (increased MMP13, ADAMTS4 and TIMP1 and decreased TIMP2 and TIMP3) in comparison to age and site-matched normal tissue. Additionally, an altered collagen II/collagen I ratio suggested a de-differentiated state, while cell proliferation appeared to be reduced, and decreased GAG synthesis was observed. Isolated cells thus had a distinct phenotype, and while some normal response to chondrogenic stimuli could be induced, a full normal response could not be elicited. This study therefore indicates that chondrocytes involved in Osteochondritis Dissecans display some facets of a permanently altered abnormal phenotype and behaviour.

The final speaker of the session was Professor Hideaki Nagase (Kennedy Institute, Imperial College, London), who gave an informative overview of the role of aggrecanases (ADAMTS4 and 5) in OA. Given the importance of aggrecan breakdown in progression of OA, targeting this disease manifestation may provide a method for effective treatments. Clinical trials with synthetic aggrecanase inhibitors however, have produced undesirable results due to non-specific inhibition of other MMP family members that are required for normal tissue homeostasis. Alternative approaches were described, whereby an understanding of the way in which MMPs interact with their substrates and other binding partners, could lead to advances in clinical treatments.

Indeed, it is known already that MMPs have different cleavage site patterns, and contain various recognition domains. ADAMTS4 for example, has no aggrecanase activity without the non-catalytic C-terminal domain, indicating that interactions with factors other than aggrecan play a key role in its function. In further support of this, full-length ADAMTS4 cleaves at one region of aggrecan, while a domain deletion mutant cleaves at an additional site; thus non-catalytic domains are involved in regulating MMP activity. It is also known that ADAMTS5 is a more efficient aggrecanase than ADAMTS4; domain swapping experiments indicated that while the catalytic domain of ADAMTS5 was more efficient than ADAMTS4, the non-catalytic domains of ADAMTS5 were also essential for this more potent activity. It was also discussed that the spacer domain of ADAMTS4, which interacts with heparin, is responsible for competitive inhibition of ADAMTS4 interaction with the aggrecan GAG moiety, while in the case of ADAMTS5, the cysteine-rich region is primarily responsible for binding extracellular matrix components. Thus soluble heparin for example, would specifically inhibit ADAMTS4 activity over ADAMTS5. It was suggested that targeting the non-catalytic domains of MMPs therefore might provide greater specificity and would provide a good basis for future therapeutic efforts. Additionally, as discussed by Ngee Han Lim, mutants of the endogenous MMP inhibitor, TIMP3, have been identified which inhibit aggrecanases but not MMPs. In a mouse model of OA, wild type TIMP3 overexpression partially protected against disease development, however the N-TIMP3 mutants were more effective, since unlike the wild type protein, they did not interfere with tissue homeostasis. Evidence was also presented indicating that the extra alanine-containing N-TIMP3 mutants might distinguish between MMPs and ADAMTSs since they cannot gain access to the active sites of the former, but can still insert into the slightly deeper active site pockets of the ADAMTSs.

Session 4: “BSMB Society Session”

This session allowed members to present research not necessarily linked to the cartilage theme of the meeting. The first speaker was Professor Karl Kadler from the University of Manchester. He presented work on tendon development, stem cell differentiation to tenocytes and the organisation of collagen fibrils. During early development, the

organism is composed of approximately 80% cells and 20% ECM, which is reversed in adulthood. Collagen fibrils, a key component of many ECMs, are tightly organised in a three-dimensional structure. In the tendon, fibrils can be over 1 cm long and must be parallel in arrangement for tissue function. Professor Kadler's group have identified structures termed fibropositors – protrusions from the cell, stabilised by the actin cytoskeleton, which are responsible for laying down collagen in a parallel pattern. Polarisation of these cells is very important and this role is fulfilled by the actin cytoskeleton, ultimately dictating tissue architecture. He has shown that cleavage of procollagen can occur intracellularly, and fibrils 20 μm long can exist inside the cell. Fibrocarriers (cables of polarised actin cytoskeleton within the cell) may also be responsible for alignment of these fibrils. Interestingly, the fibropositors have only been observed during embryonic development, at day E13.5 to E14.5 in the mouse, and in a range of tissues such as periosteal bone, heart valve, ovary and embryonic skin. Furthermore, MSCs have been found to differentiate down a tendon lineage and form fibropositors in the presence of tension alone. To study fibropositors *in vitro*, a 3D fibrin hydrogel culture system was developed, as adherence of cells to tissue culture plastic causes a loss in polarity. This model demonstrated that fibropositors have no role in the intracellular processing of procollagen and tensile force is necessary to achieve the parallel organisation of fibrocarriers, fibropositors and therefore fibrils. Professor Kadler hypothesised that procollagen is secreted from the cell and is recognised by a cell surface receptor, causing a fibropositor to form around it and tensile force to pull it to ensure the fibril is straight and in the correct orientation. Work in the chick led to a further hypothesis that a mechanical stimulus causes binding of the integrin $\alpha 5\beta 1$ to bind fibronectin, which then binds collagen, forming it into fibrils and giving rise to the fibropositor around the fibril. Questions still remain as to how the cell releases the collagen fibril once it is in place.

The next speaker was Professor John Scott from the University of Manchester, with his presentation “Extracellular matrices are held together by elastic glycan strings. Molecular details of their collagen fibril anchorages.” Collagen fibrils are known to be linked together by proteoglycans such as decoran, a fundamental role in ECM throughout

the organism. However, disruption of these interactions may have aetiology in diseases such as OA. Decoran specifically interacts with collagen by the latter's d and e bands, whilst other proteoglycans interact with collagen's a and c bands. There are 11 amino acids in these regions which are unique to the collagen molecule, and have mirror-image counterpart residues on the protein backbone of decoran, termed decoron. It was initially postulated that a single molecule of the horseshoe-shaped decoran interacts with two collagen fibrils but further x-ray crystallographic and computer simulations suggested interdigitation between collagen and decoran. The GAG chains of decoran hold the collagen fibrils together in register, while the decoron protein holds the collagen molecules together, with at least 3 collagen molecules interacting with a single decoran molecule. As decoran has previously been observed as a dimer, it was hypothesised that one half of the dimer is exchanged for collagen fibrils in the above model.

Dr Andrew Marson from the University of Manchester spoke about the development of a prototype "sugar chip" to analyse GAG-protein interactions. GAGs are a heterogeneous groups of repeating disaccharide units, with sulphation patterns varying between GAGs. They fulfil important roles in a wide range of biological processes and can interact with growth factors or cytokines to enable receptor binding, concentration gradients of factors and so on. However, there is a lack of assays to analyse GAG-protein interactions. Dr Marson described the use of cold plasma polymerisation to coat a surface with allyl amine, which then non-covalently immobilises GAGs. To optimise the percentage of allyl amine on the surface, he used the heparin-binding protein TSG-6, which can also bind dermatan sulphate, chondroitin sulphate and hyaluronan (HA). Across the range of immobilised GAGs, surfaces coated with 60-80% allyl amine showed the greatest interaction of GAGs and TSG-6. The value of the assay system was demonstrated by adding Complement Factor H, which is selective for certain GAGs, and versican, which was believed to only interact with HA. The binding patterns across a range of immobilised GAGs reflected the previously known binding capacities of these proteins. Moreover, the assay revealed a previously unknown interaction of dermatan sulphate with versican. This highlights the use of this assay as a means of identifying and

characterising interactions of GAGs and proteins with a degree of flexibility. Currently the technology is being transferred from the microtitre plate to an affymetrix-style chip.

Dr Julie Huxley-Jones from GlaxoSmithKline (Harlow) spoke about drug discovery in the ECM. Although the ECM has been implicated in the pathogenesis of a whole spectrum of diseases including schizophrenia, it has traditionally been ignored as a target by pharmaceutical companies, merely viewed as an obstacle for drugs to pass through to reach cells. She explained how the complexity of the ECM arose during evolution through genome duplication and how echoes of this are seen in conserved regions between proteins and high levels of homology within gene families. She went on to highlight how these protein components of the ECM can be targeted by a variety of means, such as with antibodies, MMPs and small molecules targeted against a particular domain. The MMPs share highly similar binding sites, so small molecule inhibitors can hit a number of MMPs, requiring less of the drug and making them polypharmable. The ECM is easily targetable and therefore is slowly gaining more recognition in the pharmaceutical field. It is responsible for the microenvironment of the cell and changes to the ECM impact on the cell; some conditions exhibit an aberrant ECM as part of the pathology and reconfiguring the matrix may overcome inappropriate ECM interactions contributing to the disease.

The final speaker in the session was Professor Ernst Hunziker from the University of Bern, with his talk “engineering of articular cartilage: alchemy of the 21st century?” He highlighted that by definition, tissue engineering must be a multi-disciplinary field, between the engineers creating the new materials and the scientists providing information and an understanding of the condition. The hypothesis behind cartilage engineering is that therapeutic repair of articular cartilage defects results in the relief of symptoms, reestablishment of joint functionality and delay in the progression of OA. One example of a success story is that of BMP-2 in bone tissue engineering, that provides patient benefit and commercial success but took from its initial identification in 1965 over 35 years to reach the clinic following FDA approval in 2002. It was speculated that new treatments would take longer to reach the clinic, if at all. For instance, autologous

chondrocyte implantation (ACI) was first described in 1987, in humans in 1994, but more recent studies have shown no improvement in cartilage repair compared to microfracture techniques developed in the 1950s. Moreover, the technique of ACI is problematic, requiring complicated surgery and expanding chondrocytes in culture whilst limiting dedifferentiation. So a new approach was taken using stem cells in combination with growth factors and a scaffold. However, considerations regarding the animal model must be made. Smaller animals cannot replicate the size of lesion found in humans and using larger animals over a longer timescale does not allow analysis of repair in the first few days. Furthermore, the act of implanting the material itself is risky – suture sites crack and cause OA-like changes in the surrounding matrix. Is the material stiff enough? This is another factor that may contribute to failure. To gain a clearer understanding of mechanisms for repair, it is important to consider the natural growth of cartilage during development and to appreciate how progenitor cells are programmed to generate the different types of cartilage. Using a systematic approach, Professor Hunziker is investigating chemotactic agents to recruit cells from the surrounding synovium and differentiate them appropriately. As the microenvironment plays a major role in cell phenotype, he has investigated explant cultures compared to micromass cultures and has found that agents such as dexamethasone have different effects depending on the culture system. BMP-2 and TGF β 1 can induce chondrogenesis but foetal bovine serum can interfere with BMP-2; BMP-2 also stimulates terminal hypertrophy of chondrocytes which is detrimental to repair. In combination with TGF β 1, however, BMP-2 can stimulate chondrogenesis but hypertrophic markers are reduced. Using synovium-derived cells a more hyaline-type cartilage can be synthesised, as these are the cells required for endogenous articular cartilage formation in the juvenile. The above factors are considerations in the future direction of articular cartilage repair.

Session 5: “*In vitro* and *in vivo* models of osteoarthritis”

This session opened with a plenary lecture from Professor James Williams (Rush University Medical Centre) who discussed “Experimental Models of Osteoarthritis”. Professor Williams began by highlighting the limitations in using human tissues for

studying complex diseases such as OA. Difficulties include standardising and grading pathological changes, quantifying disease duration, defining results at serial timed intervals and the lack of suitable controls. As such animal models present more appropriate methodologies for the study of the disease allowing defined time of disease induction, critical assessment of disease severity, progression on a detailed temporal basis and the use of controls. From the industrial perspective models are chosen to best measure the effect of medicinal compounds, with decisions made on if OA model data is required and if so is there a gold standard compound that can be used to benchmark the models. For basic and clinical sciences, human OA pathology is regarded as a common endpoint of multiple pathogenic pathways which can not be fully resembled by any animal model. Choice of model is dependent upon the scientific question being asked and takes into consideration the speed of OA development, if molecular targets between human and animal are the same and if compound metabolism is the same. Professor Williams went on to discuss examples of animal models used in the study of OA, categorising them into spontaneous models, surgically induced models and other models with OA features. Spontaneous models of OA include mice, guinea pigs and Syrian hamsters, where OA occurs most frequently in the knee joints. Canine models are affected at the hip joints in association with hip dysplasia whereas spontaneous OA in equine models is occupation related. Non-human primates have also been used as spontaneous OA models. The spontaneous OA STR/ort mouse model develops OA lesions at 12 weeks in the medial tibial plateau. The histology of the disease resembles human OA and MMP- and aggrecanase-generated neoepitopes are present within the tissue. A major advantage is the low cost and space required to house the animals when compared to larger species. However, the cause and timing of lesion development is unknown. Assessment of disease progression is largely restricted to histology with collection and analysis of serum and urine samples difficult; in addition, experimental study times are limited to less than 12 months. Male Dunkin Hartley guinea pigs spontaneously develop OA within the medial compartment, early changes are observed at 16 weeks or less and all animals have moderately severe disease by 10 months. The features of guinea pig OA are similar to those found in human including histology and collagenase profiles, OA-related biomarkers and associated risk factors such as obesity

and high bone turnover. Another advantage is the existence of a less OA susceptible control (strain 13) for comparative studies. However, the lack of genetic analysis and in particular genomic and proteomic sequence data limits the analyses that can be carried out. Canine models are particularly advantageous since serial arthroscopy is possible and physical examinations and exercise protocols can be employed. The increased size of the animal, in comparison to rodents, introduces a broader spectrum of analyses that can be used, for example radiography, CT, QCT, nuclear scans and MRI. Moreover, the tissues are sufficient for cell isolation and culture, histological, biochemical, molecular, genetic and proteomic analysis, aided by the availability of synovial fluid, blood databases and the publication of the canine genome sequence. Nevertheless, canine models are very expensive and the quadruped kinetics and kinematics differ from humans. Non-human primates are excellent models for studying OA since they share a similar history and pathology to humans. Genetic information on the rhesus macaque is available and the model has been well characterised by MRI and biochemistry. However, there is a lack of synovial effusion and the expense and availability mean that this model is not readily accessible. Professor Williams went on to discuss surgically induced animal models. He started by stating that traumatic joint injuries are regarded as antecedents to OA and that surgical injuries to the ligament and meniscus differ from clinical trauma in that the injury is direct to a specific structure or area. Rodent models are the first choice due to size, cost, and availability of transgenic animals which permits the study of specific gene effects on disease progression. OA is induced by surgical transection of the cranial cruciate ligament (equivalent to the anterior cruciate ligament in humans), meniscectomy or meniscal tear. Mild disease is observed at 4 weeks with moderate disease after 8 weeks and as previously discussed rodent OA shares common features with human OA although analysis is heavily reliant on histology. The rabbit anterior cruciate ligament transection (ACLT) is a well characterised model applied to animals older than 7 months. At 4 weeks post-surgery increased cartilage thickness, fibrillation and ulceration is observed with 50% of the animals having articular cartilage fibrillation/ulcerations after 8 weeks. The model is reproducible, making it suitable for drug testing but the animals require delicate handling by trained personnel, joint lavage is necessary to obtain synovial samples and arthroscopy is not possible. Surgically induced canine models involve cruciate ligament

transection. The timeframe of disease progression is dependent upon the activity levels of the dog allowing the effects of exercise on disease progression to be monitored. Molecular alterations can be seen as early as 2 weeks post-surgery, medial pathology by 12 weeks and cartilage erosion at 12 months. The canine model is suitable for imaging studies, repeat arthroscopy, repeat physical examination, gait analysis, sampling for biomarkers, histopathology and cell and molecular studies. However, the cost of the model as well as the quadruped kinetics of the animal would question the suitability as a model for studying the efficacy of certain OA interventions. Sheep and goat models involve cruciate ligament transection or meniscectomy with fibrillation and partial depth erosion being observed 12 weeks post surgery. Full depth erosion is seen after 6 months and osteophytosis between 3 and 6 months. The OA pathology mimics that in the human and the size of the animal permits arthroscopy, the acquisition of quality radiographs, synovial fluid aspiration and broad spectrum analyses including histopathology, biochemistry and gait analysis. Animals of the desired age are readily available as is the use of sex-matched animals with uniform genetic backgrounds, though the cost of animal husbandry is high and quadruped kinematics is a disadvantage. The first equine model discussed by Professor Williams involves osteochondral fragmentation producing OA after 72 days; repair is seen at 12 weeks, pathology at 12 months and articular erosion by 54 months. In the second model, full thickness articular cartilage defects are created on the lateral and medial femoral condyles. Because of the size of the animal a full range of biological parameters can be used for clinical assessment, including radiography, MRI, synovial fluid and serum biomarkers, gross pathology, histopathology, histochemistry and biochemistry. Disadvantages to equine models include the very dense subchondral bone creating difficulty during surgical procedures and the high cost of animal upkeep. The final part of Professor William's talk focused on the other models used by his laboratory and in particular intra-articular injections including iodoacetate, fibronectin fragments and chymopapain. Injections of iodoacetate impact on the glycolytic pathway of chondrocytes and result in loss of proteoglycans, cell loss, fibrillation and the formation of osteophytes. Aggrecanase and MMP-generated catabolic neoepitopes can be detected within the tissue with lesions being pronounced in central, habitually loaded regions. Locomotor activity is rapidly, transiently decreased in a dose-dependent manner

and this correlates with degenerative changes. The iodoacetate model is used in therapeutic intervention studies and in pain models as a methodology for understanding cartilage injury and repair. Physical intervention studies with the iodoacetate model have shown that immobilisation ameliorates chemically-induced articular cartilage damage and temporary immobilisation can facilitate repair of chemically-induced articular cartilage injury. Conversely, exercise stimulates osteophytes and diminishes fibrillation after chemically-induced articular cartilage injury. Pharmacologic intervention studies have shown that benoxaprofen can prevent fibrillation and the formation of osteophytes after articular cartilage injury, in addition trimacinelone hexacetonide can protect against fibrillation and osteophyte formation following chemically-induced articular cartilage damage. Typically, iodoacetate is used as a pain model since clinical scores of joint pain and use have been shown to correlate closely to the grade of histological findings. A reduction in peak vertical load bearing is evident 2 weeks post-injection and pain-related behaviour has been observed in two models of OA. The fibronectin fragment model was developed from observations that fragments are present in cartilage and synovial fluid of OA and RA joints whereas under normal conditions they are not. Single intra-articular fibronectin fragment injections cause reversible loss of matrix proteoglycans, production of MMPs, transient synovial effusion and anabolic effects in contralateral knees. With multiple intra-articular injections there are more severe changes leading to synovial membrane inflammation and effusion, which may be only partially reversible. The final model to be discussed by Professor Williams was chymopapain. Injections of 0.2 mg chymopapain into the rabbit knee cause enzymatic matrix insult but is followed by successful repair of the tissue within 4 weeks. In contrast, there is no successful repair following injections of 2 mg chymopapain and progressive degradation of the articular cartilage begins as early as 21 days after injury. This model has been used in a series of studies to show that levels of serum keratan sulphate are increased following the degradation of cartilage proteoglycans, pyridium crosslinks are increased in the urine of rabbits following intra-articular injections of chymopapain and that rabbit knee articular cartilage repair following proteoglycan degradation is stimulated by passive motion. In conclusion, Professor Williams discussed the criteria for the use of animal models in studying OA. The choice of model must take into consideration previous research on the

animal, the cost and ease of experimentation. Also, the ease of handling, the supply and availability of the animals and experimental techniques, anatomical/physiological attributes, the resemblance to human tissue, the availability of controls, the life span of the animal and the phase of the process to be studied. The scientist must also reflect on the evaluation methods employed. Clinical evaluation would include physical examination, gait analysis, kinematics, arthroscopy, imaging and biomarker analysis. Tissue evaluation comprises macroscopic and microscopic analysis, molecular biology techniques and biomechanical analysis.

The second speaker of the session was Gareth Edwards (Cardiff University) who talked about “enhanced nuclear localisation of Sox9 in chick limb bud mesenchymal cell aggregates levitated in an ultrasound trap”. Mr Edwards began by explaining that chick limb bud mesenchymal cells are used in micromass cultures as an *in vitro* model when investigating the developing condensation at the initiation of chondrogenesis. However, this model is flawed since some cells retain contact with the artificial substrate, leading to the formation of actin stress fibres, flattening of the cells and a decrease in SOX9 expression. Previous work in their laboratory had developed a technique called ultrasound standing wave trap (USWT) whereby the application of a sonic wave caused the formation and levitation of 2D cell aggregates which were then independent of contact from the surface substrate. Mr Edwards presented a time-lapse video clip showing that cells aggregated within 30 seconds of USWT and stated that at 30 minutes post-USWT the cells had fully adhered to each other. Immunolabelling for cell-cell adhesion molecules present within the chondrogenic condensation showed that N-CAM was upregulated within 5 minutes of USWT whereas N-cadherin was upregulated later at 30 minutes. When cells isolated from stage 23 chick limb buds were cultured on artificial substrate for 3 hours they flattened, formed actin stress fibres and did not express SOX9. In contrast, 2D aggregates that had been levitated by USWT did not form actin stress fibres and were positive for nuclear SOX9 protein. Mr Edwards went on to show that when cells that had been plated onto coverslips were exposed to an equivalent ultrasound regime they did not express SOX9 protein at the 3 hour time point, indicating that SOX9 nuclear localisation was not a direct result of ultrasound exposure. These results led Mr

Edwards to conclude that USWT promoted homogeneous cell-cell contacts without any cell-substrate interactions which would lead to the formation of actin stress fibres and the prevention of chondrogenic differentiation. The USWT technique allows rapid formation of 2D cell aggregates a controlled manner and it is anticipated that scaling-up the technology will allow enable the creation of large cartilage constructs that can be used therapeutically

The third presentation of the session was from Angus Wann (St Georges, University of London) who discussed “signal pathways that contribute to movement-stimulated hyaluronan secretion in synovial joints *in vivo*”. Mr Wann began his presentation by stating that hyaluronan (HA) is responsible for controlling hydraulic permeability of the synovial membrane and maintaining hydrodynamic joint lubrication. HA secretion into the joint is stimulated by movement and *in vitro* studies have shown that this is mediated by a Ca^{2+} -PKC γ -pERK pathway. The aim of this study was to investigate this and other related pathways on movement-stimulated HA secretion *in vivo*. Endogenous HA was removed by knee joint lavage from anaesthetised rabbits. Drugs were administered every 30 minutes by intra-articular injection with vehicle control injected into the contralateral knee joint. The cannulated knee joints were given a regime of intermittent passive cycling (0.5 Hz for 3 minutes, rest for 12 minutes) for 5 hours after which HA was harvested and analysed by HPLC. Mr Wann’s data showed that HA secretion was significantly increased with movement over the control static joint. HA secretion halved when the PLC inhibitor, U73122, was applied but had no effect on the static joint. HA secretion doubled with treatment with phorbol ester and this effect was blocked by the PKC inhibitor, bisindolylmaleimide (BIM), and the MEK-ERK inhibitor, U0126. However, when BIM and U0126 were applied to movement-stimulated knee joints without phorbol ester there was no significant reduction in HA secretion. Treatment with the calcium ionophore ionomycin increased HA secretion by 106% and treatment with phosphatase inhibitors calyculin and vanadate increased HA secretion in static joints by up to 25%. Mr Wann concluded from his results that intracellular calcium, PKC and ERK were all able to increase HA secretion and that this was dependent upon PLC activation and not PKC-

ERK activation as had been shown *in vitro*. Furthermore, his results demonstrated that HA secretion by static synovium was regulated by kinase/phosphatase activity.

Rebecca Harrison (Cardiff University) presented her data on “comparison of gene expression of human chondrocytes in monolayer and 3D-culture.” Miss Harrison began by explaining the effects of prolonged cell culture expansion on the chondrocyte phenotype. In monolayer culture chondrocytes undergo dedifferentiation causing an increase in proliferation rate, a flattening of the cell leading to a fibroblastic morphology and loss of key markers such as SOX9 and collagen type II. Dedifferentiation can be reversed by placing the chondrocytes into 3D agarose cultures resulting in the acquisition of a rounded cell morphology, increased synthesis of collagen type II and decreased proliferation rate. Whilst many studies have focused on the mechanisms involved in dedifferentiation and subsequent redifferentiation over periods of several weeks, Miss Harrison stated that very few had investigated the very early changes in chondrocyte biology that occurred as the cells switched from monolayer to 3D culture. Data from over 25 years ago had shown that rabbit chondrocytes seeded in agarose culture had a transient decrease in DNA, protein, proteoglycan and collagen synthesis and Miss Harrison aimed to investigate the gene expression changes which were occurring during this period in human chondrocytes obtained from normal and osteoarthritic cartilage. Chondrocytes were isolated from normal cartilage post-mortem and from osteoarthritic cartilage following total knee replacement surgery and expanded in monolayer. At passage 2 chondrocytes were either plated in monolayer or seeded in 3D agarose gels, cultured for 36 hours in medium with serum and then 30 hours in medium without serum. As expected Miss Harrison found that chondrocytes cultured in monolayer were flat and fibroblastic in morphology and expressed numerous actin stress fibres. In contrast, chondrocytes that had been cultured in 3D agarose were rounded in morphology and had punctate actin staining. Gene expression analysis using RNA Taqman arrays revealed increased expression of TGF β 1 and MMPs 2 and 14 in 3D agarose cultures compared to monolayer, whereas ADAMTS5 expression and ColIA1 gene expression were decreased. Genes associated with chondrocyte hypertrophy and osteogenesis were downregulated in 3D agarose cultures, as were those encoding matrix proteins. In general, the fold changes

in gene expression patterns observed between monolayer and 3D agarose culture were similar between chondrocytes obtained from normal and osteoarthritic cartilage. The notable exception to this was the gene expression profile of SOX9 which was seen to increase when normal chondrocytes were placed into 3D agarose culture and decrease in osteoarthritic chondrocytes under the same culture conditions. Miss Harrison concluded that her data correlated with previous studies showing that the switch of monolayer to 3D agarose culture was coupled with downregulation of matrix synthesis. In addition, her results have shown that the osteoarthritic pathology does not compromise the redifferentiation programme of chondrocytes, though it is proposed that the kinetics of this mechanism may be slower due to the observed decrease in SOX9 expression.

The session closed with a plenary lecture from Dr Rocky Tuan (National Institute of Health) entitled “Chondroregulatory signals in osteoarthritis”. Dr Tuan’s presentation began describing the disease burden of OA. Cartilage tissue engineering encompasses the understanding of how a cell’s properties are modulated during expansion in culture, the suitability of particular scaffolds and matrices, the biocompatibility and biomechanical properties of the engineered construct and the consideration of influence from local environmental factors, growth factors and cytokines. Adult mesenchymal stem cells (MSCs) are characterised by self-renewal in culture and multiple lineage commitment mediated in part by cross-talk between the canonical Wnt pathway controlling proliferation and non-canonical Wnt signalling influencing cell differentiation. Experiments carried out in Dr Tuan’s laboratory have shown that MSCs which have been terminally differentiated into one cell type (osteoblasts, adipocytes or chondrocytes) are able to undergo transdifferentiation in culture into one of the other two cell lineages when given the appropriate cues. In addition, terminally differentiated cells have been shown to dedifferentiate back into multipotent MSCs following removal of the differentiation stimuli and multiple passages. In order to confirm that the transdifferentiation potential of the cells arose from terminally differentiated MSCs rather than differentiation of any undifferentiated cells within the population, MSCs were transfected with a GFP reporter gene driven by the osteocalcin promoter as a late marker of osteoblast differentiation. Transfected MSCs were differentiated under pro-osteoblastic conditions for 15 days and

sorted for GFP expression by FACS. GFP-positive cells representing 5% of the total cell population were able to differentiate into chondrocytes and adipocytes demonstrating conclusively the transdifferentiation potential of the cells. Dr Tuan postulated that the processes of MSC differentiation and dedifferentiation were regulated by specific gene sets which could be identified by microarray analysis and investigated further using techniques such as RT-PCR, siRNA and forced gene expression. Identification of these genes and their function in self-renewal and the control of lineage differentiation will provide understanding as to the role of the stem cell niche and the environmental regulation of MSC biology *in vivo*. Of the genes identified during the microarray analysis Dr Tuan discussed the role of actin filament associated protein (AFAP). AFAP was found to be highly expressed in undifferentiated MSCs, downregulated during differentiation and then re-expressed during the process of dedifferentiation. Studies involving siRNA showed that cell proliferation was reduced following AFAP knockdown and osteogenesis and chondrogenesis were both inhibited. Furthermore, culture of MSCs with recombinant laminin $\alpha 4$ chain showed increased proliferation, decreased adipogenesis and increased osteogenesis which was proportional to laminin concentration. Dr Tuan went on to say that the importance in understanding the molecular mechanisms of MSCs self-renewal and multilineage differentiation was evident in the recent data involving induced pluripotent stem (IPS) cells in which pluripotent ES-like cells could be generated through transfection of four transcription factors: OCT3/4, SOX2, c-MYC and KLF4. The second part of Dr Tuan's talk focused on the integration of MSCs into poly- ϵ -caprolactone (PCL) scaffolds. MSCs had an increased proliferation rate when seeded onto electrospun PCL nanofibres compared to microfibrils and this technology was scaled up using a bioreactor to create large constructs of between 4-6 mm. The centre of a piece of native cartilage was cored out, replaced with the engineered cartilage construct and histological analysis carried out to show that full integration of the two tissues had taken place. Dr Tuan progressed to discussing the *in vivo* integration of the constructs using a mini-pig model. Full thickness defects were created in the animal joint, loaded with MSCs and nanofibre scaffold and demonstrated capacity to repair the tissue. Dr Tuan went on to conclude that this technology could be optimised further so that cell attachment and alignment within

the scaffold allowed the engineered tissue to transmit loads and also translated to meniscal repair strategies.

Session 6: Advances in Treating Osteoarthritis

Dr Peter Van der Kraan (Radboud University, Nijmegen) started the session with an outline of some of the current approaches used in treating OA, such as physical therapy and surgical intervention. It was discussed that effective treatment should aim to restore the balance between catabolic and anabolic mechanisms, and regulate deranged chondrocyte differentiation. It was also highlighted that there are many similarities between OA and the ageing process, which might further our understanding of this disease. Understanding the failure of previously unsuccessful approaches targeting catabolic and anabolic mechanisms might also provide insights. In the case of catabolic activities, blocking IL-1 activity had little effect on OA, while MMP-13 inhibition resulted in side-effects due to non-specific inhibition of other MMPs. The DDR2 receptor, which binds to collagen fragments and upregulates MMP13, could provide a new target to block MMP13 activity upstream. Introducing anabolic factors such as IGF-1 and BMPs to stimulate matrix production were also unsuccessful due to unwanted side effects in the case of the latter, while IGF-1 had no effect presumably due to high levels of endogenous IGF-1. To modulate chondrocyte behaviour, understanding signals associated with development and differentiation might provide a basis for future directions. Changes in Wnt and WISP signalling pathways in synovium and cartilage through from development to ageing were discussed and some evidence presented to validate WISP1 as a possible target. Signalling pathways associated with TGF β were also discussed in the context of age-related changes. Signalling by this growth factor through different ALK receptors activates alternative Smads to differentially stimulate or block differentiation. With age, levels of ALK5 are reduced more quickly compared to ALK1, thereby favouring the Smad pathways associated with ALK1, which promote differentiation and degradative pathways. A further understanding of development and ageing in terms of the switch from temporary to terminally differentiated cartilage could therefore be utilised to support future therapeutic directions. It was also noted that OA is

a disease that has developed without any evolutionary pressure, due to greater precedence amongst individuals who have passed the reproductive age, highlighting the crucial role played by the ageing process in development of this disease.

Dr Linda Troeberg (Kennedy Institute) considered the mechanisms by which a chondroprotective agent, calcium pentosan polysulphate (CaPPS), protects against OA. CaPPS is a highly sulphated polysaccharide that has been shown to block aggrecan breakdown through multiple mechanisms, with some similarities to those observed with another highly sulphated polysaccharide, heparin. Evidence was presented to suggest that interaction between ADAMTS4 and 5 and aggrecan was blocked by CaPPS through competitive interaction with the non-catalytic domains of these aggrecanases. Secondly, CaPPS inhibits endocytosis of the endogenous aggrecanase inhibitor TIMP3 through the low-density lipoprotein receptor related protein, thus inhibiting degradation and resulting in increased protein levels. Thirdly, it was suggested that TIMP3 affinity for ADAMTS4 and 5 is enhanced by CaPPS, and that this observation was dependent on the presence of the non-catalytic domains of the ADAMTSs. It was also shown that CaPPS requires the presence of TIMP3 to inhibit OA progression, thus supporting the role of TIMP3 as the principal effector which is modulated by CaPPS in this disease.

Caroline Curtin from the National University of Ireland presented data to support the use of MSCs in developing effective cell-based strategies for treatment of OA. To validate the use of MSCs, evidence was presented that these cells can be retained in cartilage, and furthermore, that retained cells exhibit chondrocyte-like behaviour. Fluorescently labelled (GFP) MSCs were added to cartilage biopsies which had been isolated from damaged cartilage and it was shown that GAG synthesis was enhanced and further, that the GAGs were retained in this model. It was also shown that the MSCs were capable of infiltrating into fibrillations in the tissue and producing collagen type II in addition to GAGs. Infiltration and adherence of MSCs was further investigated to determine an optimised protocol, and was found to be dependent on factors such as time, cell seeding density and the level of cartilage fibrillation.

Dr Helen Roberts (Cardiff University) presented a novel approach to improve existing treatments for OA patients. Glucosamine is used to contribute to successful management of OA, however very high doses are required to see any benefit, and furthermore the cellular mechanisms responsible are unclear. This study investigated chemically modified glucosamine as an approach to enhancing the efficacy of this molecule. A modified glucosamine compound, glucosamine-C6-monophosphate (Glu5), was found to be a bioactive molecule, which importantly had no greater toxicity in MTT assays than unmodified glucosamine. The lipophilicity of this molecule imparts membrane-soluble properties which may contribute to its enhanced activity in inhibiting IL-1 induced proteoglycan degradation in a bovine explant model. It was demonstrated that production of aggrecanase-generated aggrecan fragments was reduced in the presence of Glu5 compared to unmodified glucosamine. Induction of ERK activation by IL-1 was also reduced by Glu5. This glucosamine derivative therefore inhibits cytokine-induced proteoglycan degradation by inhibiting ERK activation and aggrecanase activity. This model provides a basis for continuing studies to screen modified glucosamine compounds in order to identify potent inhibitors of aggrecanase activity and highlights the possibility of modification of existing approaches to improve future treatment of OA. Further, this study may elucidate some of the mechanisms of action of glucosamine, which ultimately could lead to new therapeutic targets.

The closing speaker of the meeting was Dr Carl Flannery from Wyeth Research in Cambridge, USA, giving us insights into the biological mechanisms and potential treatments in the pathogenesis of OA. As we understand more about the pathogenesis of OA, potential therapies targeting the MMP and ADAMTS families are under consideration. In healthy cartilage, two molecules are important in reducing friction in the joint – aggrecan and water. In response to pressure and shear forces, aggrecan can release water to enable lubrication. In 1959 Professor Sir John Charnley first realised that synovial fluid was not the main lubricant on the articular surface and Eric Radin and David Swann subsequently identified lubricin, a glycoprotein also known as surface zone protein and megakaryocyte stimulating factor. It consists of two somatomedin-B (vitronectin-like) domains, a heparin-binding domain, a mucin-like domain and a

repeating motif of KEPAPTT residues. It exists in synovial fluid as a multimer and is located on the articular surface and to a depth of 2-3 cells; lubricin also prevents cell adherence to the joint surface and loss of cartilage matrix from the surface. Depletion of lubricin results in joint pathology; camptodactyly-arthritis-coxa vara-pericarditis syndrome (CACP) occurs when lubricin is not secreted. In addition, knock-out mice develop hyperplasia and tendon abnormalities, resulting in OA. Whilst lubricin supplementation would be beneficial in treating joint disease, synthesis is difficult due to the mucin-like domain. Dr Flannery has removed two thirds of this domain and termed the resultant protein "LUB:1". Following expression in CHO cells and isolation from the medium, the novel protein retains the ability to form dimers and undergo glycosylation. LUB:1 can bind to the cartilage surface and friction testing revealed that increasing LUB:1 up to 25 µg/ml increased lubrication. By increasing LUB:1 concentration, cell adhesion to plastic could also be prevented. LUB:1 was further assessed in the rat meniscal tear model (with a histological scoring system) over 6 weeks, and following supplementation after 1 week on a weekly basis, 20 µg significantly reduced degeneration, the total joint score, the degeneration width and the width of severe lesions. Moreover, there was no evidence of fibroplasia, which can occur following anti-MMP treatment and LUB:1 was easy to inject due to its non-viscous properties. LUB:1 now needs to be tested in a large animal model and the frequency and concentration of the dose optimised. The efficacy in more advanced OA will be assessed, as will the efficacy of a second generated protein with a larger mucin-like domain. The future work on this protein may reveal a potential candidate in the treatment of OA.