

“The Multi-dimensional Matrix” Newcastle University Medical School
September 5 and 6th, 2006

The Autumn BSMB meeting “The Multi-dimensional Matrix” was organised by Drs Drew Rowan and David Young from the Newcastle Musculoskeletal Research Group. Held in the Newcastle Medical School on September 5 and 6th, the meeting attracted an excellent turnout with 115 registrants of which over 50 were students. There were 40 non-members, and we would encourage everyone to take up membership and enjoy the benefits of being part of the BSMB. This meeting was co-ordinated with the Oliver Bird Rheumatism Programme which held its annual meeting immediately prior to the BSMB so as to enable the students to attend. Newcastle is one of the five centres within the UK currently participating in the Oliver Bird PhD studentship scheme. The meeting included no less than 8 international speakers who were complemented admirably by the excellent UK-based speakers. Sponsorship for the meeting made this possible, and the organisers gratefully acknowledge the support of Astra Zeneca, the Company of Biologists, Devro, IDS, Perbio, Tebu-bio, VWR, as well as the Oliver Bird Rheumatism Programme. The BSMB reporter bursaries of £125 each went to Matt Barter, Matt Farren (both at Newcastle) and Emma Blain (Cardiff), whilst a conference presenter bursary of £125 was awarded to Olajumoke Adeniji (Sheffield). Elizabeth Crawford (Manchester) also received £125 to support her attendance at the meeting as the current student representative on the BSMB committee. All students should note that this is a benefit given to student representatives once during their one year term of office – we hope that it will encourage students to get more involved in the running of the society. The posters and selected short talks were all of a high standard, and the International Journal of Experimental Pathology-sponsored poster prizes (£150 each) were awarded to Kate Silverthorne (Manchester), Lise Berg (Copenhagen) and Lorna Fiedler (Cardiff).

The meeting’s first session entitled ‘Matrix gene regulation’ saw insightful and thought provoking presentations from various researchers. Matt Vincenti (Dartmouth Medical School) opened the session with a presentation entitled “IL-1 activation of matrix metalloproteinase-1 (MMP-1) transcription in chondrocytes.”

Matt’s work focuses on the signalling mechanisms behind the induction of MMP-1 by IL-1 β . IL-1 has been known for many years to be a key cytokine involved in the pathogenesis of various arthritic diseases. The presence of IL-1 in the joint leads to the increased expression of MMP-1, which in turn leads to the breakdown of collagen type II and irreversible cartilage damage. Matt’s hypothesis suggests that nuclear factor kappa (NF- κ B) and extracellular signal-regulated (ERK) signalling pathways could be involved in IL-1-dependent expression of MMP-1. To investigate this, Matt has stimulated human articular chondrocytes (HACs) and the chondrocytic carcinoma cell line SW1353 with IL-1 β at 10ng/ml for 24 hours in the presence of various inhibitors of signalling pathways, and MMP-1 gene expression assayed with real-time PCR. IL-1 induction of MMP-1 was comparable in HACs and SW-1353s, both temporally and quantitatively. Matt has used various techniques, such as RNAi and dominant negatives to inhibit different aspects of the NF- κ B signalling pathway. SiRNA specific to RelA, a subunit of the active NF- κ B protein, showed a large reduction in MMP-1. SiRNA to NF- κ B1 and NF- κ B2, proteins involved in the inhibition of NF- κ B signalling, augmented MMP-1 expression. These data clearly suggest that NF- κ B is involved in the induction of MMP-1. Inhibition of the ERK pathway via small molecular weight inhibitors has also demonstrated the importance of this pathway in MMP-1 expression. Matt has also carried out transfection and reporter assay studies of the MMP-1 promoter, identifying an IL-1 β responsive region with a consensus CCAAT enhancer-binding protein (C/EBP) binding site. The ERK inhibitor was found to decrease the phosphorylation of C/EBP on threonine 235, which activates the transcription factor. This demonstrates that there may be a novel role for C/EBP in the regulation of MMP-1, and that IL-1 β mediates its effects on MMP-1 via the NF- κ B and ERK signalling pathway.

The second talk, based on the poster entitled “The contribution of connective tissue growth factor (CTGF, CCN2) to fibroblast biology,” was presented by Dr Andrew Leask (CIHR Group in Skeletal Development and Remodelling, University of Western Ontario). Andrew’s work focuses on the molecular mechanisms of CCN2 and its role in tissue remodelling by fibroblasts during development. Genome wide expression profiling of CCN2^{-/-} embryonic fibroblasts suggests that CCN2 is required for the basal expression of over 490 mRNAs and the induction of 345/943 TGFβ responsive mRNAs. Also, experimental evidence from hierarchical clustering and bioassays suggest that fibroblastic migration, matrix production and adhesion are all dependent on the growth factor CCN2. Signal transduction and transcription factors which mediate CCN2 action were also investigated by dominant mutant studies, pathway inhibitors and siRNA. The data collected from these studies confirmed the requirement of CCN2 in the TGFβ-dependent activation of focal adhesions kinase, Akt and ERK. The data suggested that CCN2 is a cofactor of TGFβ signalling, rather than a downstream component of the pathway. Andrew’s data provide a new insight into the role of CCN2 as a growth factor required for tissue remodelling by fibroblasts during development.

The third talk in this section was presented by Simon Tew (UK Centre for Tissue Engineering, University of Manchester) and was based on the poster entitled “IL-1β activation of p38MAPK in human osteoarthritic articular chondrocytes increases SOX9 expression and is dependent on cytoskeletal organisation.” SOX9 has been shown to be a key regulator of many cartilage extracellular matrix genes and is often used as a chondrocytic phenotype marker. Simon has previously shown SOX9 mRNA stability was dependent on the p38 MAPK pathway. More importantly, this effect was only seen when actin stress fibres were disrupted in the cell. In his recent work he has been looking at the direct effect of IL-1β on the effect of mRNA for SOX9. Human articular chondrocytes (HACs) were cultured with inhibitors for protein synthesis, ROCK1, 2 (actin stress fibre inhibitor) and a p38 inhibitor. SOX9 expression was then analysed via real time PCR. The data obtained suggested that SOX9 expression was not affected by IL-1β. However, the inhibitor studies suggest that a combination of stress fibre inhibition and IL-1β treatment in dedifferentiated HACs at 5 hours resulted in a p38 dependant increase in SOX9. These data reveal some of the more complicated mechanisms of SOX9 mRNA regulation in chondrocytes.

This session was ended by a presentation from Prof Derek Mann (Liver Research Group, Newcastle University), entitled “The role of NF-κB in the progression and regression of fibrosis.” Liver fibrosis was once considered to be a one way progressive incurable disease. However, recent experiential evidence has suggested that disease process is highly dynamic and can both progress and regress. Recent clinical observations have seen successfully spontaneous regression of livers once the underlying insult had been successfully removed. There is also an increasing body of evidence which suggests that the life span of wound healing hepatic myofibroblasts (HM) has a direct balance of progression and regression. HMs in the insulted liver derive from the trans-differentiation of resident hepatic stellate cells and perisinusoidal fibroblasts. HMs are positive contractile cells that promote the net deposition of collagen I/III rich ECM via their excretion of TIMP-1 and collagen. Experimental evidence suggests that HMs undergo apoptosis during the switch from fibrotic progression to fibrotic regression. Therefore, there has been a lot of interest in the molecular mechanism behind this switch and the resulting apoptosis of HMs. Derek’s work has been focusing on the transcription factor NF-κB, as it has been shown to be highly expressed in HMs. He has shown that pharmacological inhibitors such as gliotoxin and sulfasalazine, along with molecular inhibitors of the IKK/NF-κB pathway will promote HM apoptosis. Moreover, studies of models of fibrosis reversion have shown that these inhibitors will stimulate regression, which is also associated with reduced expression of hepatic TIMP-1 and elevated MMP activities. Derek more recently has been trying to exploit HM-specific features of NF-κB to develop novel pharmacological and molecular inhibitors aimed at promoting the reversal of fibrosis in patients. This targeted approach should allow a treatment of liver fibrosis to be developed.

The second session “**Matrix Development and Disease**”, chaired by Prof Carl Richards (McMaster University, Canada), was opened with a presentation by **Prof Steve Goldring** (Harvard Medical School, Boston, USA) entitled “*The role of cell-matrix interactions in osteoclast differentiation: you are what you eat*”. Bone remodelling is a highly dynamic process involving osteoclast activation, resorption to remove the mineralised tissue, followed by reversal to allow the osteoblasts to invade and form new trabecular bone. The question posed by Professor Goldring was what determines the commitment pathway to a particular lineage? - hematopoietic stem cells are able to differentiate into dendritic cells or macrophages as well as osteoclasts. There are key markers of particular lineages: for example, during differentiation into osteoblasts RANKL, M-CSF, IL-1, IL-6, IL-7, IL-15 and TNF α are expressed, whereas differentiation into a macrophage lineage promotes expression of GM-CSF, IFN β and γ , IL-4, IL-10 and IL-13. Another question was posed to determine how macrophages differentiate into the correct lineage i.e. how a macrophage knows whether it is synovial or alveolar. Professor Goldring’s studies indicate that the cells are capable of correct differentiation due to adaptation to their microenvironment and the signalling mediators presiding in the tissue i.e. via cell-cell interaction, soluble cell products, extracellular matrix components or growth and differentiation factors. Two pathologic models of bone loss of interest to Professor Goldring are rheumatoid arthritis (RA) and peri-implant osteolysis. One of the phenotypic changes in RA is synovial inflammation brought about by synovial hyperplasia, neovascularisation, loss of mineralised tissue and subsequent bone erosion. This removal of the mineralised matrix in RA results from the activities of the residing osteoclasts; cells lining the bone at the site of resorption have several phenotypic markers including multinucleation, and the presence of proton pumps, $\alpha_v\beta_3$ integrin, cathepsin K, TRAP and calcitonin receptor. The precursor for the osteoclasts must exist in the tissue or in the vascular system where signals from the bone matrix regulate osteoclast function at the bone surface. Such a mechanism has been analysed by Professor Goldring’s lab in peri-implant osteolysis after joint replacement; the primary cause of joint replacement surgery failure is the development of areas of osteolysis at sites of fixation. An observation that cyclic loading of total hip replacement causes the release of polyethylene from the cover of the implants has since been demonstrated to promote foreign body granuloma, due to infiltration of macrophages and enhanced inflammation. A comparison of the phenotype of cells interacting directly with the bone surface or with those interacting with the implant polymer showed that both subsets had increased levels of TRAP and cathepsin K, but cells associated with the polyethylene implant did not express calcitonin receptor – indicative of a distinct bone phenotype. Murine calvarial discs were implanted subcutaneously or intra-murally and it was shown that at the cut edges of these discs there was a granuloma inflammatory response (both TRAP and calcitonin receptor positive). The cut edges of the bone discs were resorbed via an osteoclast-mediated mechanism, which was not apparent at the non-cut edges. An involvement of the MMPs at the resorptive sites on the bone surface was hypothesised. Using specific inhibitors of the collagenases, a reduction in bone resorption by the residing osteoclasts was observed, confirming previous evidence in which a reduction in bone resorption was noted in a collagenase cleavage site-mutant mouse. Therefore, calvarial discs pre-treated with collagenase I prior to implantation initiated a mass influx of inflammatory macrophages at the bone surface. Further, collagenase treated calvarial discs were cultured with RANKL/M-CSF on either plastic or calvarial bone, and microarray analysis performed. The super-induction of the calcitonin receptor with respect to TRAP was found in osteoclasts plated on a bone surface leading to the idea that the ratio of calcitonin receptor: TRAP can be used as a marker of terminal differentiation. Another marker of osteoclast terminal differentiation discussed by Professor Goldring was annexin VIII, which is found in cells lining the bone surface but is not present in associated macrophages – a localisation pattern observed in resorbing sites in RA bone. One of annexin VIII’s many functions is in the organisation of the actin cytoskeleton via modulation of Ca²⁺ signalling. Annexin VIII over-expressing cell lines treated with RANKL induced the formation of giant osteoclasts, whereas in annexin VIII siRNA treated cells an abnormal osteoclast morphology was observed; this altered morphology was attributed to a failure of the actin rings to form on a dentin substratum. The presentation was completed by the conclusion

that the bone substrate provides “signals” to the osteoclasts that are essential for terminal differentiation into specialised tissue-specific bone macrophages. Pictorially, this mechanism was compared to a man (colony stimulating macrophage) influenced by his environmental cues i.e. dietary habits at McDonalds (bone substratum) morphing into an obese version of his former self (osteoclast).

Dr Kate Silverthorne (University of Manchester) presented novel data on “*C-1-1 as a potential therapeutic gene to promote cartilage repair*”. ERG – Ets-related gene is a transcription factor of which there are 3 family members (ERG1, 2 and 3). ERG-3 is involved in promoting endochondral ossification in the chick growth plate/developing limb; however, a splice isoform of the ERG-3 gene exists termed C-1-1 (in which exon 6 is missing), which has previously been demonstrated to arrest chondrocyte hypertrophy and mineralisation. After injurious damage, articular cartilage is unable to effectively repair and treating osteoarthritis has become a therapeutic challenge to researchers of the wider connective tissue community. During the early stages of osteoarthritis there is a reparative attempt by the tissue itself, but this recapitulation often leads to differentiation of growth plate-like chondrocytes. Dr Silverthorne has hypothesised that blocking the process of endochondral ossification in articular chondrocytes may be a potential therapeutic target. Thus the objective of her study is to transfect chondrocytes with C-1-1 to prevent differentiation into hypertrophic chondrocytes, such that this population of cells may ultimately be utilised in autologous chondrocyte implantation. Little information of these genes in mammalian systems is available, therefore primers were designed to the specific ERG isoforms, and transcript levels determined during mouse limb development. At embryonic stage E13.5 and E17.5 ERG-2 and -3 were preferentially expressed, whereas C-1-1 was more highly expressed in adult chondrocytes. Dr Silverthorne also examined differential expression of the 3 ERG isoforms in an *in vitro* model of endochondral ossification using the ATDC5 growth plate chondrocyte cell line, where addition of insulin promotes a hypertrophic phenotype. Using quantitative PCR, C-1-1 expression was observed during early stages of differentiation concomitant with increased type II collagen expression. During later stages of chondrocyte hypertrophy, a decrease in C-1-1 mRNA was observed correlating with an increase in type X expression. The data indicates that C-1-1 may maintain the articular chondrocyte phenotype, and that its use in gene therapy may assist in preventing inappropriate differentiation of chondrogenic cells applied to injured cartilage. Currently Dr Silverthorne is transducing the ATDC5 cell line with C-1-1 or ERG-2/-3 to determine whether hypertrophy can be arrested.

Rose Davidson (University of East Anglia) presented her data on “*Expression profiling of metalloproteinases and their inhibitors in synovium and cartilage*”. Previous studies from the University of East Anglia have characterised the expression profile of matrix degrading enzymes: matrix metalloproteinases (MMPs) and the enzymes from the “a disintegrin and metalloproteinase domain with thrombospondin motifs” (ADAMTS), and the tissue inhibitors of MMPs (TIMPs) in osteoarthritic (OA) and normal articular cartilage. Although cartilage destruction observed in osteoarthritis is driven by the chondrocyte, low-grade synovitis is also a hallmark of this disorder. Therefore, Rose’s postgraduate studies have concentrated on characterising the gene expression profile of MMPs, cytokines and growth factors in normal and osteoarthritic synovium to try and identify genes that may contribute to the pathology of osteoarthritis. RNA was extracted from synovium and cartilage obtained at joint replacement surgery, reverse transcribed and analysed by quantitative PCR. MMP 28, ADAMTS16 and 17, and TIMP 2 were the most significantly upregulated genes in OA synovium; interestingly there is a correlation as MMP 28 and ADAMTS 16 were also observed to increase in diseased cartilage. There was a significant reduction in MMP 10, ADAMTS 4 and ADAMTS9 in OA synovium. Again, the reduction in MMP 10 and these ADAMTS are co-ordinately regulated in both the synovium and cartilage of OA patients, suggesting similar modes of regulation. Rose has also utilised Taqman low-density microarrays (covering 269 cytokine or growth factor genes) on the OA tissue. Of note was the coordinate down-

regulation of IL-6, the IL-6 receptor and the associated gp130 in OA cartilage. Likewise, genes involved in RANK signalling were also co-ordinately regulated in OA – both RANK and RANKL were elevated concomitant with a down-regulation of the RANK antagonist OPG. Rose Davidson's comprehensive analysis of genes that are differentially regulated in OA synovium has demonstrated that there is an overlap in genes expressed in OA synovium and cartilage showing a coordinate regulation of genes involved in matrix degradation in several components of the synovial joint.

The session was closed by **Professor Mary Goldring** (Harvard Medical School, Boston, USA) discussion of “*Novel mediators of matrix remodelling during cartilage development and osteoarthritis*”. Osteoarthritis is characterised by focal lesions, coincident with tidemark duplication, cell proliferation, apoptosis, and subchondral bone changes. Of the MMPs, the primary mediator of articular cartilage collagen degradation is MMP 13, and Professor Goldring discussed 3 mediators that regulate MMP 13 expression:- ESE-1, GADD45 β and DDR2. The MMP 13 promoter is well characterised and of the known DNA elements are several potential ESE-1 sites (GGAA). Using promoter analysis, MMP 13 induction by IL-1 β was shown to be via ESE-1 sites; using EMSA one consensus site was observed to mediate the highest binding with contributions from two adjacent tandem ESE-1 sites. Another mediator of MMP 13 induction discussed by Professor Goldring was GADD45 β , an anti-apoptotic molecule - abrogation of GADD45 β mRNA expression, using siRNA, was shown to enhance TNF α -induced apoptosis. The bone morphogenetic proteins (BMPs) are recognised as important signalling molecules involved in the process of chondrogenesis. Using microarray analysis, treatment of cartilage chondrocytes with BMP2 for 1 hour was shown to increase GADD45 β by 3.8-fold. In the developing mouse (stage E15.5) GADD45 β expression was observed in pre-hypertrophic chondrocytes, and further was shown to co-localise with Runx2. The Runx2 knockout is characterised by short stature, compression of the hypertrophic zone, decreased expression of MMP13 and type X collagen mRNA with a concomitant reduction in mineralisation. Therefore, Runx2 has been shown to play a crucial role in hypertrophic differentiation. Using epiphyseal chondrocytes in a 3-dimensional pellet culture system, BMP2 was shown to regulate MMP13 expression via SMAD1/Runx2 mediated induction of GADD45 β . Professor Goldring hypothesised that GADD45 β may be involved in cartilage chondrocyte catabolism and hypertrophy – key features of OA. This was confirmed using array analysis of early versus end stage OA where GADD45 β expression was elevated in early OA, and reduced in late OA along with a reduction in cell cycle-associated genes. Additionally, it was shown that a decrease in GADD45 β expression correlated with increasing severity of OA (as assessed by the Mankin score); staining was localised predominantly to chondrons in early OA cartilage. Immunolocalisation studies demonstrated a nuclear distribution of GADD45 β in normal cartilage, whereas in early OA the protein was localised in mid and deep zone chondrons. Localisation was confined to deep zone hypertrophic chondrocytes in end stage OA cartilage, which was associated with tidemark advancement/endochondral ossification. The final molecule discussed by Professor Goldring was discoidin domain receptor 2 (DDR2), the expression of which is induced by native type II collagen. In the *cho* mouse – generated by a loss-of-function mutation in type XI collagen and characterised by an OA-like phenotype, there is increased expression of DDR2 in association with increased degradation of the triple helical region of type II collagen. Plating of chondrocytes onto type II collagen led to increased expression of both DDR2 and MMP13, however MMPs 1, 3 and 8, and ADAMTS 1 and 2 were not affected, indicating a specific effect on MMP13. Interestingly, Professor Goldring demonstrated that in the *cho* mouse (> 2 months of age), expression of GADD45 β was localised to the deep zone cells only and that ESE-1 was also elevated in the cartilage chondrocytes. From these studies, it was speculated that in early stages of OA, ESE-1 and GADD45 β are highly expressed and as the cartilage changes towards end stage OA DDR2 expression is elevated. The presentation was concluded by recognising that divergent and convergent signalling pathways are involved in the balance of Ets, AP-1 and Runx2 transcription factors which determine the extent of MMP13 promoter activity allowing distinct temporal and spatial induction of MMP13 in articular cartilage.

In the morning of the second day Prof Tim Cawston introduced the “Matrix Proteomics” section commencing with Prof Dick Heinegard’s presentation entitled “Diverse post-translational modifications of extracellular matrix proteins regulate functional properties”. He began by highlighting the roles of post-translational modifications in providing a fixed charge density, guiding interactions with charge, providing direct interactions with proteins thereby regulating matrix assembly and stability, and binding growth factors and cytokines. With regard to cartilage structure and function he detailed how chondrocyte receptors bind matrix components thereby signalling to cells to produce or repair matrix components. One type of modification allows Leucine rich repeat (LRR) proteins in the ECM such as biglycan, decorin and fibromodulin to bind collagen via interactions with cationic domains in the LRRs. These domains can also bind basic domains such as those of chondroadherin and PRELP forming bridges between collagen. Another type of posttranslational modification occurs in the tyrosine sulfate domain of molecules like fibromodulin, lumican and osteoadherin. For fibromodulin this allows it to modulate collagen fibrillogenesis *in vitro* and has been found on collagen fibrils *in vivo*. Dick’s work has involved the generation of fragments of the N-terminal domain of fibromodulin, discovering they act like heparin in their ability to bind proteins containing heparin binding motifs e.g. MMP13, PRELP, b-FGF. The extent of the charge density dictates how much binds thereby providing specificity of interactions. In disease there is a progressive destruction of the organisation of the matrix, what with the loss of aggrecan first, then fibromodulin and collagen last. Dick’s group have found that IL-1 can induce specific cleavage of the fibromodulin tyrosine-sulfated domain in cartilage breakdown, and that MMP13 can cleave, with more than one cleavage site. Such cleavage would release any bound growth factors or cytokines with resulting implications.

A poster talk by David Robinson followed, entitled “Developing a new way of investigating glycosaminoglycan:protein interactions: the “Sugar chip””. He detailed the aims of his PhD with a view to a greater understanding of the interactions between sugars and other macromolecules. Using heparin as a model, he has been trying to optimise its adsorption onto microtitre plates plasma-polymerised with allylamine, with X-ray photoelectron spectroscopy for analysis of the extent of ligand binding. By creating heparin gradients across the plates he has identified that binding is dose responsive and dependent on incubation time and the medium within which the heparin is contained. It was important to determine whether the heparin retains its structure once bound and is still functional. Thus, using a heparin binding protein TSG-6, they found that it was indeed able to bind the heparin adsorbed onto the plates.

The final talk of the morning session was by Prof Christopher Overall entitled “CLIP-TAILS and CLIP-STEP: New degradomic techniques for the discovery of natural substrates and proteases in complex cell proteomes”. With his well-honed public speaking charisma and mastery of PowerPoint slide transitions Chris championed the CLIP-TAIL proteomic approach that they have developed to quantitatively identify the cleaved substrate degradome in order to identify novel substrates *in vivo*. Using CLIP-TAILS labelling and MS/MS they were able to identify a wealth of new natural substrates for various MMPs, with the technique able to extend to other classes of protease. Another proteomic approach is able to assay shedding and degradation through the measurement of cleavage products, after transfection of proteases into the cell environment. Here a multiplex analysis involving iTRAQ labelling and MD-LC MS/MS is used to distinguish relevant substrate cleavage events from indirect effects of proteolysis. For example the effects of MMP-2 at 3, 24 and 48hour were looked at. A process of “Peptide Mapping” attempts to even locate the cleavage sites by analysis of the cleaved peptides and their abundance. He explains that indirect effects of proteolysis can also be determined with multiplex iTRAQ experiments, such as effects of proteolysis on cellular signalling. He hopes to use all these techniques to fully analyse the

interrelationships between proteases, inhibitors, cofactors, receptors and their substrates, shedding light on the “protease web”.

The next session “The Matrix and Inflammation” chaired by Prof Ian Clark began with Gerry Graham’s talk on the “Regulation of the inflammatory response by the chemokine receptor D6”. Introducing chemokines, with their cysteine hallmark, he detailed their role in the migration of leukocytes, via the binding of 7 TM domain receptors. These receptors are promiscuous in terms of ligand binding repertoire, but similarly they must accommodate unfaithful ligands. Of interest to Gerry is the chemokine receptor D6 which is highly promiscuous, able to bind all inflammatory CC chemokines, and atypical with regard to unusual expression sites, for example barrier tissues and placenta. By virtue of a mutation in its signalling peptide the receptor doesn’t seem able to signal, and thus it is likely a decoy receptor, which can internalise ligand in an independent manner and drop it into lysosomes. To analyse D6 in vivo, they created a D6 null mice which is healthy and fertile. Using TPA (phorbol ester) skin painting as a model for cutaneous inflammation, they determined that chemokine drainage from inflammatory sites is delayed in the KO mice, meaning that they cannot clear away the mess after inflammation. Increased chemokine presence results in a psoriasis-like cutaneous pathology with upregulated markers. This can be blocked with anti-TNF therapy, for TPA upregulates chemokine transcription through TNF signalling. But, Gerry asks, what are the implications for other pathologies? Using a DMBA/TPA skin-painting model for the induction of cutaneous tumours, they found the KO mice exhibited increased tumour burden and increased susceptibility, implicating D6 as a classic tumour suppressor gene. Thus they wondered whether D6 could be overexpressed for therapeutic use. Driven by a keratin 14 promoter, transgenic mice expressing D6 do indeed have a delayed onset of tumours and decreased burden. He finished by postulating whether there might be less D6 in cancer susceptible strains of mice.

Second up was Christopher Buckley from Birmingham with his work on “Defining a role for fibroblasts in the persistence of chronic inflammation”. He began by asking what the relationship is between inflammation and repair, why inflammation is site specific, why leukocytes accumulate and what determines the switch to persistent inflammation???? With regard the time, place and cell type he stressed “duration, duration, duration” with regard to the need to kick in anti-inflammatory processes as well as remove those pro-inflammatory. As for “location, location, location”; compartmentalisation and a combination of chemokine receptors deliver cells to the right ‘postcode’. The stromal area-code is defined by fibroblasts. Importantly different chemokines are expressed in physiological and pathological inflammation, for instance embryonic cytokines are re-expressed in pathology. Fibroblast populations change in disease, with important consequences as fibroblasts, both typical (peripheral) and atypical (lymphoid), make not only matrix but a range of chemokines too, with a pattern of expression depending upon location e.g. skin, rheumatoid. These fibroblasts exhibit positional and topographic memory which is *hox* gene dependent. Chris wondered whether the regional identity of fibroblasts could be modified, finding that stimulation with TNF could induce typical and atypical cells to change cluster. But, he asks, what are the functional consequences? Taking fibroblasts from skin, bone marrow and synovium, he identified site specific differences in their ability to keep T cells alive and in their activation. Thus, is leucocyte adhesion to endothelium regulated by fibroblasts? Certainly synovial fibroblasts seem able to increase adhesion.

The Matrix and Inflammation session was finished by Prof Carl Richards (Department of Pathology and Molecular Medicine, McMaster University, Canada), presenting a talk entitled “The role of oncostatin M (OSM) in tissue remodelling.” OSM and other cytokines have been shown to be involved in pathological progression of inflammatory diseases. OSM is known to synergise with

other cytokines, such as IL-1 and TNF- α , to exacerbate joint destruction in murine models of arthritis. OSM is also part of the IL-6 cytokine family which has also been implicated in the pathological progression of arthritis. The receptor accounts for some of the redundancy seen in this family. Carl's recent work has focused on the signalling pathways utilised by OSM in connective tissue. He has focused on how these signalling pathways result in the unique biological activities seen with OSM, which are distinct from other IL-6 family members. Carl has used immunoblots, immunoprecipitation and kinase analysis to show that OSM signalling pathways include STAT5, STAT6 and PKC- δ . He has also presented data which suggests the OSM receptor is critical for signal transduction. He has also shown that OSM receptor $-/-$ mice show decreased strength of male mouse femurs. There was also a dramatic increase in the adipocyte population in the bone marrow of aging female $-/-$ mice. OSM has been shown to dramatically decrease the differentiation of 3T3-L1 preadipocytes to adipocytes. OSM also induces osteoblast-like phenotype in mesenchymal cells derived from mouse adipose tissue. These data suggest a role for OSM in the homeostatic bone integrity in mice as well as its additional role in inflammatory diseases.

The final session of the meeting, chaired by Prof Chris Overall regarding "Models in Matrix Biology", proceeded with Amanda Fosang's presentation "Type II collagen resistant to collagenase cleavage at Gly775 \downarrow 776Leu: Preliminary characterisation of the collagen II knockin mouse". She began by outlining the paradigm of aggrecan loss first or collagen clipping first to allow aggrecan loss. Then detailing the characteristics of MMP knockout mice, she noted that both the MMP-13 $^{-/-}$ and MMP-9 $^{-/-}$ mice have delayed endochondral ossification with extended growth plates, MMP-8 $^{-/-}$ and MMP-2 $^{-/-}$ mice have normal development but impaired inflammation or angiogenesis respectively, while the MMP-14 $^{-/-}$ mouse suffers dwarfism, osteopenia and arthritis. Upon introducing the Type I collagen knockin mouse, which is normal until older when it develops osteopetrosis, skin fibrosis and impaired involution of the uterus. She highlighted the differences between knockins and knockouts, detailing that a knockin is a mutation in a substrate, thus there is no compensation by other enzymes if one were knocked out, and related to this the fact that only collagenolysis will be affected rather than other substrates. Thus, Mandy and her collaborators developed a collagen II knock-in mouse with the identical mutation, which was resistant to MMP-1, -8 and -13 digestion. There were no obvious differences between the normal and knockin mice. Upon further analysis the newborns have elongated growth plates with delayed secondary ossification centres and mineralisation, which doesn't resolve after 3 weeks unlike the MMP-13 $^{-/-}$. At 8 weeks both fore and hind-limbs are significantly shortened (30% shorter femurs), and at 26 weeks the joints are a highly abnormal club shape, prohibiting arthritis studies. Preliminary in vitro aggrecanolysis and collagenolysis studies show passive aggrecan loss is reduced by 50%. Mandy proposed two mechanistic possibilities to explain the knock-in phenotype. The first suggests an excess of unresorbed collagen II forms a barrier interfering with chondrocyte hypertrophy and mineralisation, while the second hypothesis involves the lack of generation of bioactive collagen fragments involved in regulating endochondral ossification.

The second talk of the session was by Alan D. Murdoch (University of Manchester), based on the poster entitled "Improved chondrogenesis of human bone marrow mesenchymal stem cells (MCS) in transwell cultures." Alan's research focuses on deriving chondrocytes from mesenchymal stem cells obtained from the bone marrow. Chondrogenesis has been shown to occur in MSCs when they are grown in pellet aggregates and stimulated with specific growth and differentiation factors. However, these cells show none uniformity and have limited transplant applications. Recent work by Alan has made advances in the area of tissue culture techniques in order to improve the cell uniformity and transplant viability of chondrocyte cultures. MSCs were obtained by adherence to tissue culture plastic and were expanded in mono-layer culture. At P3 cells were trypsinised and transferred to chondrogenic medium. Aliquots of cells were centrifuged in either 15 ml tubes to form aggregate culture, or in Transwell filter units (discs of 6.5mm diameter made of up a polycarbonate membrane with pore sizes of 0.4 μ m). Cells were then cultured under identical

conditions and their chondrogenesis assessed by Real-time PCR and matrix synthesis and deposition. The disc cultures showed a more uniform chondrogenic phenotype with a greater net wet weight when compared to aggregate culture. They also had a 50% greater GAG/DNA composition of the matrix than the pellets. Taken together this data suggest that the new protocol provides a more efficient form of culturing chondrocytes from MSCs and may one day provide a new technique for creating viable cartilage for transplantation.

The penultimate presentation of the session was made by K.A. Piróg-Garcia (The University of Manchester). The presentation was entitled “Genetic background influences the severity of growth plate dysplasia in a mouse model of pseudoachondroplasia.” Pseudoachondroplasia is a skeletal dysplasia which results in short stature and early onset of osteoarthritis. Pseudoachondroplasia results from a single mutation of a 500kDa pentameric glycoprotein found in the ECM called COMP. Pseudoachondroplasia exhibits both inter- and intra-familial variability between affected people. This suggests that there are different genetic modifiers at work in each case. The identification of these differences may help build a possible molecular mechanism for the disease and thus provide a possible target for a new therapy. A current murine model of pseudoachondroplasia, generated from a T583M mutation on a CBA/57BL/129Sv mixed background were crossed with wild type C57BL/6 mice to generate a congenic strain. The heterozygous mice resulted in a more severe form of the phenotype. This genotype cross had shorter tibia and a reduced ECM when compared to the mixed background. The data suggests that the breeding of the COMP mutant mouse on to the C57BL/6 background results in an increase of the severity of chondroplasia. This new breed of mice can be used in the study of the genetic modifiers which are different in the various forms of chondroplasia and may lead to the development of novel therapies.

Prof David Eyre closed the meeting with a lecture on “Cartilage Collagens: Highly evolved heteropolymers with tissue-specific chain assemblies and post-translational modifications”. He began by introducing the collagen types in hyaline cartilage where type II can constitute >90%, while type III and IX can exist around the 10% area. Collagen IX is found covalently linked to the collagen II fibril surface where it is a molecular modifier of nascent collagen II fibrils. It is the most cross-linked collagen, and must be cleaved to grow the fibrils out in the inter-territorial space. Similarly collagen XI is also very crosslinked and covalently bonded with collagen II, although also found with collagen V. Together, collagens V and XI are an essential adaptable template for collagen fibrillogenesis. Collagen III is also found linked to collagen II, however this is deposited in adults as a covalent modifier of the type II fibril network. In summary, intertype collagen cross-linking is common in matrix biology. The fibrocartilage of the vertebral disc is a further example of the diversity in collagen forms and connections, where collagen V/XI hybrid molecules and the short form of collagen IX determine a gel-like collagen II network. Interestingly there seems to be a different distribution of collagen V/XI between growth and maturity, for example with collagen XI making way for collagen V in hyaline cartilage, perhaps in the process mapping out the way. David wonders whether collagen V/XI control fibril size and organisation. Finally, he spoke about the long known 3-hydroxyproline residue near the C-terminus of each α -chain of collagens I and II. It has been found to be lacking in the crtap-null mouse, leading to a skeletal phenotype with abnormal growth plates and severe osteoporosis.