

British Society for Matrix Biology – Sprint 2011 Meeting Report



Advances in Musculoskeletal Repair and Regeneration

Written by Natasha Agabalyan, Matthew Mayhew and Henry Jia

The BSMB spring meeting of 2011 was hosted by Bristol University, on Monday the 11th and Tuesday the 12th of April. The meeting, organised by Dr Wael Kafienah, with the support of Jane Lohmann, Jan Cunningham and Professor Anthony Hollander, was held in the Wills Memorial Building for both talks and poster sessions and was attended by around 130 delegates including speakers.

The meeting comprised of 5 sessions, each focusing on one particular tissue in the musculoskeletal unit: Bone, Cartilage, Tendons and ligaments and Skeletal and cardiac muscle, with the addition of an Open Session. The prestigious Fell-Muir Award (sponsored by the International Journal of Experimental Pathology) was presented to Professor Bruce Caterson of Cardiff University for his work on the Glycobiology of GAGs. A talk by Dr Michael Patnick, Head of Research and Education at Arthritis Research UK highlighted what the body had to offer in way of fellowships and funding opportunities.

Reporter Bursaries were awarded to Miss Natasha Agabalyan (Brighton and Sussex Medical School), Mr Matthew Mayhew (University of East Anglia) and Mr Henry Jia (University of Bristol). Presenter Bursaries were awarded to Dr Cleo Bonnet (Cardiff University), Miss Sarah Turner (ARC, Robert Jones & Agnes Hunt Orthopaedic Hospital), Dr Raewyn Poulson (University of Oxford), Mrs Niina Hopper (University of Cambridge) and Dr Maan Al-Abbasi (University of Bristol). Several Poster Sessions were held over the course of the two days and Mr Steven Woods (Newcastle University), Dr Siyuan Li (Cardiff University) and Miss Sian Morgan (Cardiff University) were awarded Poster Prizes (sponsored by the International Journal of Experimental Pathology) by the plenary speakers.

The meeting was generously sponsored by Thermo Scientific (Platinum), The Company of Biologists, Development, The Journal of Experimental Biology, Disease Models and Mechanisms, Journal of Cell Science (Gold), National Stem Cell Network UK (Silver), Life technologies, Merck Millipore, MACS Miltenyi Biotec, PeproTech, Promega, R&D Systems, Roche, Sigma-Aldrich, Scientific Laboratory Supplies and VWR (Bronze).

Session One - Bone

Chaired by Dr Andy Pitsillides (The Royal Veterinary College) and Dr Simon Tew (University of Liverpool)

Craniofacial tissue engineering by Professor Gordana Vunjak-Novakovic (Columbia University, USA)

Session one began with an exciting presentation by **Professor Gordana Vunjak-Novakovic** (Columbia University, New York) on '*Craniofacial Tissue Engineering*'. She highlighted the importance of tissue engineering by stating that as we live longer, we need spare parts and that bioengineering helps unlock the full regenerative potential of stem cells, helping to provide the dream of 'staying forever young'. Prof. Vunjak-Novakovic stated a surprising figure that two thirds of people who have ever reached the age of 65 in the history of civilisation are alive today, with life expectancy in classical Greece a mere 28 years, with relatively recent advances in medicine bumping up this figure only since the 20th Century.

Prof. Vunjack-Novakovic went on to state that cells respond to the entire context of their environment, for example, to cytokines and extra cellular matrix (ECM), and thus we need to move on from *in vitro* petri cultures to more physiologically relevant and controllable environments that resemble an *in vivo* environment, aided by the use of various bioengineering solutions such as scaffolds and bioreactors. However, current bioengineering treatments are not completely satisfactory – for example, bone grafts are very difficult to connect to the vascular network; they sometimes require bone to be taken from another part of the body, causing local damage to the site of explantation; and they are very difficult to shape correctly.

Two alternative bioengineering solutions are the use of 'naked' decellularised bone and a hydroxyapatite (HA)-silk fibroin composite. By using either of these scaffolds, Prof. Vunjack-Novakovic described how it is possible to grow both bone and cartilage tissues in one system, by interfacing a hydrogel containing chondrocytes on top of a mineralised scaffold with human mesenchymal stem cells (hMSCs) which is under perfusion and then applying mechanical loading onto the cartilage. In their best experiment, Prof. Vunjack-Novakovic stated that they had obtained between 11-12% mineralisation by volume by culturing hMSCs in the silk scaffold. She reported that this proportion of mineralisation is a bit poor compared to normal bone, but does represent seriously osteoporotic bone, and demonstrates the osteogenic ability of HA to differentiate hMSCs, from bone marrow or adipose tissue, to produce bone without the need of growth factors (GFs), with the percentage of mineralisation proportional to the amount of mineral (HA) in the starting culture. Prof. Vunjack-Novakovic stated that the rate of perfusion is also important, with the optimal perfusion rate around 800 $\mu\text{m}/\text{sec}$, which is close to the physiological rate of perfusion. Finally, the architecture of the scaffold was also shown to be important, with smaller pores in the scaffold producing bone resembling flat bones, which are found for example in the skull, and larger pores producing more trabecular-like bones. Flow patterns were also shown to dictate bone morphology. Using various animal studies, Prof. Vunjack-Novakovic demonstrated the ability of these bioengineered scaffold tissues to heal flat bone and trabecular bone defects in a nude mouse and nude rat model respectively significantly better than various controls.

Other advantages of using these scaffolds were also emphasised over current forms of treatment. For example, a degree of vascularisation has been observed using these bioengineered scaffolds, and the use of 3D imaging was also highlighted, allowing these scaffolds to be exactly shaped according to the patient. These are two problems that still plague current conventional treatments.

Prof. Vunjack-Novakovic finished her presentation by reiterating that conditions inspired by the *in vivo* environment were necessary to utilise the full regenerative properties of stem cells. It is possible to not only to create viable, composite and functional tissue grafts, but to also create custom tissues grafts, something especially important for craniofacial tissue engineering where everybody's face is unique and thus a custom tissue graft can not only improve physical function of a body part, but also improve the psychological well-being of the patient undergoing treatment.

Bioprinting approach for regenerative medicine; towards an osteochondral implant? By Dr Jos Malda (Utrecht University, The Netherlands)

The second presentation was given by **Dr. Jos Malda** (UMC Utrecht, Netherlands) on '*Bioprinting Approach for Regenerative Medicine; Towards an Osteochondral Implant?*'. Dr. Malda began by introducing his research area looking at osteochondral grafts. He states that cartilage is made up of chondrocytes which reside in various distinct zones, each with a distinct function and so different matrix constituents can be found in these different zones. To mimic the different layers of cartilage, his team decided to utilise a bioprinting approach using a 3D fibre deposition (3DF) technique, allowing for computer-controlled precision to 'print' various 3D constructs using cells isolated from the different zones of cartilage and various biomaterials, including the use of thermo-responsive and

photo-responsive polymers. Dr. Malda went on to describe various different 3D constructs and patterns they had created and their efficacy towards expressing various different markers, including both differentiation and dedifferentiation gene markers, with different types and patterns of constructs causing expression of different markers. One challenge that was highlighted was the need to know what need there is for dictated organisation and whether it's crucial to be in a micrometre scale or whether a millimetre scale is sufficient. Dr. Malda closed by reiterating that as it possible to dictate the layered structure of these constructs, it will be possible to determine the need for the interaction between various zones and constituents.

Subchondral bone thickening, with or without articular cartilage lesions, in response to joint loading by Dr Blandine Poulet (Royal Veterinary College, London)

The third presentation of the day was given by **Dr. Blandine Poulet** (The Royal Veterinary College, London) on '*Subchondral bone thickening, with or without articular cartilage lesions, in response to joint loading*'. Dr. Poulet began her presentation by pointing out that osteoarthritis is a disease of more than one tissue, highlighting that in addition to degeneration of articular cartilage (AC), the underlying subchondral bone (SCB) has also been shown to be thickened, leading to chicken or the egg paradox of which comes first, with evidence of both – AC degeneration or SCB thickening? A murine model was used to explore the relationship and interactions between mechanical loading and genetics using mice from different genetic backgrounds. In these experiments, non-invasive *in vivo* mechanical loading of 9N was performed on the right knee of the mice 3 times a week for various time periods. Micro-CT scanning was then performed on the left (non-loaded) and right (loaded) knee joints with SCB thickness measured and analysed by CTAn software. SCB thickness was significantly increased after 5 weeks of loading in the lateral femur, with SCB thickening co-localising with AC lesions, suggesting SCB thickening occurs in response to loading immediately below AC lesions. In the lateral tibia however, there were no AC lesions but some SCB thickening after 5 weeks of loading, suggesting SCB thickening is not down to AC lesions alone. SCB thickness was also more prominent in the most posterior region of the lateral tibia, corresponding to the area which is most compressed during loading, suggesting SCB thickening is as a result of mechanical loading. In conclusion, changes in SCB thickness are induced by mechanical loading independently of AC lesions, but AC lesions can enhance load-induced SCB thickening.

New Biomaterials strategies for orthopaedic tissue engineering by Professor Molly Stevens (Imperial College London)

The final presentation of the first session was given by **Professor Molly Stevens** (Imperial College London) on '*New biomaterials strategies for orthopaedic tissue engineering*'. Prof. Stevens began by saying that most work covered in this talk was either unpublished work or had just been accepted for publication. She first demonstrated that simple biomaterials can be used for tissue engineering when used in a clever way; in an early experiment, injection of calcium alginate hydrogel with no GFs or cells under the periosteum of the tibia in rabbit, creating an *in vivo* bioreactor, resulted in the influx and proliferation of cambial cells, resulting in the growth of new bone that can be used for transplantation elsewhere in the body. Replacement of the biomaterial with hyaluronic acid (HA) containing liposomes which could slowly release Suramin to stop blood-vessel growth, creating an artificial hypoxic environment, resulted in the growth of cartilage. This demonstrated that simple biomaterials could be used for tissue engineering, and thus Prof. Stevens posed the question of how complicated should biomaterials be, with a need to strike a balance between simplicity of the biomaterial against the amount of 'information', either from the biomaterial or GFs, put in for the cells.

Prof. Stevens went on to discuss her lab's current work using bacterially-produced γ -polyglutamic acid (γ -PGA), shown to be very good at mineralising as a biomaterial. Esterifying the side-group –

COOH groups, shown not to be cytotoxic, could alter the water-solubility of the γ -PGA, allowing for use in tissue engineering. Prof. Stevens revealed that, for example, the benzyl form of the esterified γ -PGA could increase the osteogenicity of hMSCs. The team later went on to alter the mechanical properties, with help from Professor Paul Smith (ETH, Zurich), by making a film of the polymer, aligning the fibres in the film, resulting in an increase in modulus and tensile strength, which can be tailored for various types of tissues.

Prof. Stevens then discussed the use of inorganic/organic hybrid nanocomposite scaffolds, which are organised to the nanoscale covalent-linkage structure. Phase imaging revealed nicely arranged domains of around 40 nm within this covalently-crossed nanocomposite material. Interestingly, Prof. Stevens demonstrated with videos how altering the cross-links could tailor the mechanical properties of the material, ranging from very spongy materials to very tough ones that could take a fair bit of load, highlighting how these materials can be used for various tissues by tailoring the nanoscale structure.

Prof. Stevens then revealed a drug, strontium ranelate, that they have recently had approved for human clinical use, taken in multi-gram doses every day in patients to strengthen bones and reduce the probability of fracture by stabilising osteoblasts to make bone and preventing osteoclasts from resorbing bone. The team, in a material they have commercialised as StronBone™, substituted some of the Ca^{2+} ions from a bioactive glass with strontium, resulting in upregulation of osteoblast and downregulation of osteoclasts. In a sheep femur model, use of StronBone instead of control material resulted in stronger, stiffer bones with significantly less soft tissue. Prof. Stevens revealed news that they have just received funding for a 68 patient trial, which will be starting imminently in London in the hospitals affiliated with Imperial College.

Prof. Stevens finished her talk by describing how they could track cell differentiation and mineralisation over time in live cells via the use of Raman micro-spectroscopy. This provides a more in-depth analysis over the course of differentiation compared to traditional alizarin red staining for mineralisation, which only detects the amount of calcium carbonate present in the mineral in fixed cells. For example, Raman micro-spectroscopy can detect the level of crystallinity. Using this technique, in conjunction with traditional *in vitro* methods, the biomechanics of *in vitro* bone formation can be better understood and further elucidate and characterise the differences between cell-source-specific materials to facilitate the development of clinically successful engineered bone.

Session Two - Cartilage

Chaired by Professor Anthony Hollander (University of Bristol) and Dr Emma Blain (Cardiff University)

Hypoxia – a force for Good in Cartilage by Dr Chris Murphy (Kennedy Institute of Rheumatology, London)

The afternoon session opened with a last-minute change to the plenary speaker timetable with **Dr. Chris Murphy** (Imperial College London) kindly stepping in to give a presentation entitled '*Hypoxia – A Force for Good in Cartilage*' as Professor Michael Buschmann (Ecole Polytechnique of Montreal, Canada) unfortunately had to cancel his presentation on '*Therapeutic technologies using natural polymers*' due to illness. Dr. Murphy began by highlighting the importance of hypoxia in AC, an environment that AC is in constantly in larger animals due to a lack of vasculature, stating that AC uses this hypoxic state to regulate cartilage-specific gene regulation. He further went on to highlight the importance of AC in joints, to allow for near-frictionless movement and load-bearing in joints, with its two main constituents, type II collagen and the aggregating proteoglycan aggrecan containing sulphated glycosaminoglycan (GAG) side-chains, providing the cartilage with these

important properties. Dr. Murphy stated that SOX9 is the main tissue-specific transcription factor that regulates these matrix genes for cartilage-specific function.

Dr. Murphy mentioned various anabolic factors that drive cartilage-specific gene expression, including mechanical loading, GFs such as the TGF β and BMP family, and importantly, hypoxia. He showed that in human cartilage explants, a physiologically relevant oxygen tension (1-3% O₂) upregulated *COL2A1*, Aggrecan and *SOX9* mRNA, compared to non physiological oxygen tension (20% O₂). Dr. Murphy presented an overview of the Hypoxia-Inducible Factor (HIF) pathway, known to regulate gene expression during hypoxia, and demonstrated via siRNA knockdown that it is predominantly HIF-2 α and not the more characterised HIF-1 α that is responsible for hypoxia-induced *SOX9* upregulation in isolated human chondrocytes and it is this *SOX9* regulation which in turn regulates expression of Type II Collagen. The HIF proteins themselves are regulated via prolyl hydroxylase domain (PHD) proteins, with depletion of PHDs resulting in stabilisation of the HIF proteins. It was shown via siRNA that depletion of PHD2, the most abundant PHD in AC, enhanced HIF levels in both normoxic and hypoxic conditions and that this depletion of PHD2 specifically, and not PHD1 or PHD3, upregulates *SOX9*, with greater upregulation at 1% O₂ tension.

Dr. Murphy then went on to discuss the anti-catabolic effects of hypoxia, demonstrating that culturing human cartilage explants in hypoxia results in less cartilage matrix protein degradation and can also inhibit IL-1 α -mediated aggrecan degradation. Dr. Murphy then explored the role of HIFs in this anti-catabolic effect, demonstrating an upregulation of tissue inhibitor of metalloproteinase (TIMP)-3, a chondroprotective protein known to inhibit the matrix metalloproteinases (MMPs) and aggrecanases, during hypoxia and showed that this upregulation of TIMP-3 is HIF-1 α -dependent and not HIF-2 α -dependent. Hypoxia was shown to downregulate MMP13, with inhibition of HIF-1 α upregulating MMP13.

Dr. Murphy concluded his talk by reiterating that the anabolic effects of hypoxia are driven by HIF-2 α which regulates *SOX9* expression, which in turn regulates cartilage-specific gene expression. The anti-catabolic effects of hypoxia are mediated by HIF-1 α which results in an upregulation of TIMP-3 and downregulation of MMP13 and ADAMTS-5. Since both HIF-1 α and HIF-2 α are stabilised by inhibition of PHD2, PHD2 inhibition may therefore represent a potential means of inducing both cartilage repair and protect against cartilage degradation in a large animal model. Murine cartilage may not reside in a hypoxic environment due to only being a few cells thick, despite being avascular, and therefore murine cartilage gene expression may not be regulated in the same way as in larger animals.

miR324-5p in osteoarthritis and Indian hedgehog signalling by Mr Steven Woods (Newcastle University)

The second presentation was given by Ph.D student **Steven Woods** (Newcastle University, UK) on '*miR324-5p in Osteoarthritis and Indian Hedgehog Signalling*'. Mr. Woods began his talk by giving an overview of microRNAs (miRNAs) and how they inhibit gene expression by targeting mRNA for degradation, and mentioned that due to imperfect base-pairing between the miRNA and the target mRNA, one gene can be targeted by many miRNAs. Mr. Woods described how a previous group had knocked out Dicer in mice, an important enzyme involved in the processing of miRNAs from their premature state, in all cells that express type II collagen, which resulted in a severe phenotype which included a high post-natal mortality rate by the time of weaning, a relatively proportional reduction in skeletal size and enlarged hypertrophic regions. Mr. Woods went on to describe another research paper in which miR140 had been knocked out in mice which although lead to OA, resulted in a far less severe phenotype compared to the conditional dicer knockout, indicating that other miRNAs may be involved in cartilage homeostasis.

Via screening miRNAs via RT-PCR in OA and healthy cartilage, Mr. Woods discovered an upregulation of miR324-5p in OA cartilage compared to undetectable expression in healthy cartilage. He demonstrated how miR324-5p could target a protein called Gli1, a transcription factor involved in the Hedgehog (Hh) signalling pathway. In a luciferase reporter assay, co-transfection of Gli1 3'-UTR with miR324-5p in SW1353 human chondrosarcoma cells resulted in a decrease of luciferase activity, which was rescued by mutating the binding site on the Gli1 3'-UTR, indicating a direct interaction between miR324-5p and Gli1. In another experiment, addition of miR324-5p to BMP2- and Ihh-stimulated C3H10T1/2 murine MSCs abrogated an increase of alkaline phosphatase levels, a marker of chondrocyte hypertrophy and bone formation, suggesting miR324-5p may inhibit hypertrophy and bone formation by inhibiting the Ihh pathway through its interaction with Gli1, an important pathway in OA.

To investigate other potential targets, a SILAC (stable isotope labelling with amino acids in cell culture) proteomics approach was taken whereby cells were either labelled with heavy amino acids or unlabelled and subsequently treated with miR324-5p or a control miRNA respectively, and their lysates mixed. A decrease in protein expression in the heavy labelled cells compared to the non-labelled cells would indicate which genes may be targeted by miR324-5p. This was cross-referenced with various 3'-UTR target prediction algorithms to resolve new potential novel targets for miR324-5p, which will be validated by further luciferase assays.

Mr. Woods concluded his talk by reiterating that miR324-5p is upregulated during OA and has been shown to be involved in the inhibition of the Ihh pathway, an important pathway during OA, and that miR324-5p may also target various other genes.

BMP-7 in cartilage repair by Professor Susan Chubinskaya (Rush University, USA)

The final presentation of the session was given by **Professor Susan Chubinskaya** (Rush University, Chicago) on '*BMP-7 in cartilage repair*'. Prof. Chubinskaya began by giving an overview of cartilage repair, stating that although cartilage can attempt to repair itself, newly synthesised cartilage is more susceptible to re-injury compared to mature cartilage. She mentioned that the bone morphogenetic protein (BMP) family has been extensively studied in this area but that BMP-7 is the most potent in various different types of human cell culture, being the only member of the BMP family to overcome the effect of catabolic cytokines, with or without serum. Knockdown of BMP-7 using siRNA for 7 days was also shown to inhibit proteoglycan and aggrecan synthesis. Treatment of chondrocytes with BMP-7 for 48 hours, however, upregulated the expression of various chondrocyte-specific genes, including types VI and IX collagen, and these were downregulated when treated with siBMP-7 instead.

Interestingly, co-treatment with insulin-like growth factor (IGF)-1 and BMP-7 increased cell proliferation and matrix deposition compared to treatment with these growth factors separately. Blocking BMP-7 in human chondrocytes also blocked IGF-1 and IGFR expression, with an increase in MMP-13 expression. Similarly, stimulation with BMP-7 upregulated IGF-1/IGFR expression as well as various other genes implicated in IGF signalling and cell cycle genes. This suggests that BMP-7 restores responses to IGF-1 which are lost with aging chondrocytes. Co-culture of these two growth factors in Autologous Chondrocyte Implantation (ACI) chondrocytes promoted cell survival when grown in culture, which die overtime in culture without IGF-1 and BMP-7. However, co-treatment did not increase proteoglycan deposition.

Using an acute cartilage trauma method for *ex vivo* modelling, Prof. Chubinskaya noted cell death by necrosis in the area immediately below injury with the rest of the cells remaining viable. Cell death by apoptosis and matrix degradation were also observed in the early phase. At a later stage, anabolic responses were observed, including activation of superficial zone protein and autocrine BMP-7. If left

untreated, with time, cell death expanded beyond the area of trauma. Treatment with BMP-7 prevented expansion of cell death and improved tissue integrity, as measured by proteoglycan staining, with its stage of action coinciding with the anabolic stage of the untreated explants.

Prof. Chubinskaya finished her talk by discussing further work using a similar trauma-induced OA model *in vivo* in various animal models including sheep, goats and rabbits. Interestingly in an osteochondral defect model in goats, there was an autocrine production of BMP-7 in response to both trauma alone and treatment with exogenous BMP-7, as detected by antibodies against the mature form of BMP-7 used in the treatment, and the pro- form of BMP-7 produced endogenously by the cell. Similar responses were also found in various other animal models.

Prof. Chubinskaya concluded that BMP-7 has both pro-anabolic and anti-catabolic effects on cartilage, stimulating tissue repair and degeneration after trauma, improving cartilage integrity, inducing autocrine BMP-7 signalling, preventing the effects of pro-catabolic cytokines and preventing trauma-induced cell-death expansion. This therefore makes BMP-7 a key target in OA.

Fell-Muir Award - The Glycobiology of GAGs; fun for a few but a headache for some by Professor Bruce Caterson (Cardiff University)

The first day closed with the BSMB Fell Muir Award being awarded to **Professor Bruce Caterson** (Cardiff University) for his continued work on GAG biology, sponsored by the *International Journal of Experimental Pathology*. The start of Prof. Caterson's talk on '*The Glycobiology of GAGs: fun for a few but a headache for some*' was unfortunately plagued by technical issues. When Prof. Caterson was able to resume, he highlighted that extra cellular matrix is important in a number of processes ranging from metabolism to motion and motility, pointing out that if you were to remove all ECM from your body; the rest of your cells would fit into two pint glasses. Prof. Caterson stated that as modifications to the extra cellular matrix cost energy, they must have an importance. He went on to mention the sheer structural diversity of GAG modifications, with the function of many to bind to glycan binding proteins (GBPs) such as growth factors and chemokines, highlighting that there are around 4000 different combinations of GAGs through various linkages, α or β conformations, sulphation patterns, disaccharide composition, etc. Prof. Caterson entertained the floor by demonstrating various different combinations of chondroitin sulphates on his presentation slides using children's toy Duplo blocks aided by a cuddly toy koala bear acting as growth factors.

Prof. Caterson also spoke about the use of monoclonal antibodies to detect specific GAG chains. He demonstrated the ability of these monoclonal antibodies by showing toluidine blue staining of human embryos, staining the anlagen blue due to the presence of chondroitin sulphate (CS). Using monoclonal antibodies against specific GAG chains, Prof. Caterson was able to highlight specific areas of the cartilage matrix, for example, only cartilage that would go on to develop into articular cartilage rather than the rest of the developing growth plate. Antibody staining patterns were also shown to change during the stages of development, illustrating the dynamic changes of CS epitope turnover during development, which would ordinarily be hidden using standard toluidine blue or alcian blue staining. Prof. Caterson also described collaborations with other labs looking at various types of stem cells, demonstrating a change in CS-antibody staining with maturing stem cell populations, suggesting the surrounding proteoglycan matrix may protect these stem cell niches from the influence of cytokines, etc., that are binding to other GAG chains, creating GBP gradients necessary for cellular differentiation and proliferation during normal tissue development and in repair/regeneration.

Session Three - Tendons and Ligaments

Chaired by Professor Roger Smith (Royal Veterinary College) and Dr Hazel Screen (Queen Mary, University of London)

The role of matrix and cell-matrix interactions in tendon and ligament repair by Professor David Butler (University of Cincinnati, USA)

The second day of the conference was opened by **Professor Butler** (University of Cincinnati, USA) with a talk focused on understanding normal matrix function and structure of tendon and ligament. An introduction centred on the statistics of tendon and ligament injuries, the cost of rotator cuff surgeries (>250 K) and anterior cruciate ligament (75K-125K) were highlighted as well as their limited success (15-20% for the anterior cruciate ligament). The beginning of his talk covered normal matrix structure, function and development, during which he explained the difference between different tissues and the different forces that are placed upon them during daily activities, stressing that ligaments show a lower failure force than tendons. The forces that can be undergone are dependent on species and area of interest and it is possible to analyse these using IVDs as function of activity. TGF α and TGF β are found at the centre of the signalling for normal development and are regulated by mechanical stress, although the interactions between them are yet unknown. This was followed by a focus on the damage incurred by tendons and ligaments during age and the differences found here. Ageing reduces biomechanics (a rabbit will lose 25% of its tendon strength between 1 and 4 years of age) and it is known that tendon fibril diameter is reduced also. This could be due to the increased type V collagen (which regulates type I collagen). Ageing tendons are sensitive to loading and show reduced stiffness and forces as well as a 50% reduction in thickness, area and compressive modulus. When looking at the matrix distribution, there is a substantial reduction in GAGs as well as a 200% increase in permeability. Professor Butler asks the following questions: Does tendon naturally heal? What are the patterns of gene and protein expression? Are cells migrating into wound sites? Underlining work in both rabbit and Col1-Col2 transgenic murine models, the talk was concluded with current work on improving natural healing results using collagen-based scaffold matrices augmented with mesenchymal progenitor cells and mechanical stimulation in culture. Future work would involve looking into tendon normal development as well as possible stem cell populations and growth control and how to generate cultures for successful repairs of both tissue and insertion sites.

Mechanical regulation of matrix turnover in Human Tenocytes by Miss Eleanor Jones (University of East Anglia, Norwich)

A second presentation given by **Miss Jones** (University of East Anglia, Norwich) focused on the regulation of matrix components in tendinopathies. Studies in both the Achilles and the Rotator Cuff tendon in disease have revealed disruptions of the extracellular matrix homeostasis (increased in proteoglycans, matrix turnover, type III collagen) and in particular an increase of TGF β expression. Her study focuses on analysing the effects of cyclic strain loading and TGF β stimulation on protease and ECM protein expression by human tenocytes. Analysis of cell cultures by qPCR, a luciferase assay and gelatin zymography showed patterns of strain response in a large range of genes, mirrored with TGF β stimulation. It was shown that activation of TGF β was increased with strain but not mRNA expression. The talk then focused on finding the mechanism that transforms latent TGF β to active TGF β . A series of genes and signalling pathways were investigated including MMP inhibitors, RGD inhibitors, serine proteases and calcium signalling but to no avail. In conclusion it was shown that cell-cell contact is important in mechanotransduction and that mechanical strain regulates certain proteases and matrix genes. The important role of TGF β in activation was highlighted while its mechanism and role in disease remains to be discovered.

Fate of mesenchymal stem cells following different *in vivo* administration routes in repair of tendon injuries by Dr Jay Dudhia (The Royal Veterinary College, London)

The third talk by **Dr Dudhia** focused on the fate of mesenchymal stem cells in repair of equine tendon injuries, primarily centred on the tendon of the forelimb. MSCs have already been used in this way but cell survival has been shown to be low at 3-4 months after implantation. The aims of his work are to promote retention of MSCs in tendon after intra-lesional and intravenous injections. A clinical study of horses 21 days after onset was performed using randomised administration routes (intravenous, intra-lesional and regional perfusion). Although only 2.7-22.5% of cells remained labelled, this was deemed enough to continue the study. Cell persistence was shown to be at only 10% within tendons after 24 hours after intra-lesional injections. Intravenous injection showed even less detectable cells in the tendon. However, regional perfusion shows a significant labelling of the tendon. It was concluded that although the optimal number of cells effective in regenerative treatment is not known, the highest cell numbers were found after intra-lesion injections. Regional perfusion could be a possibly alternative if no core lesion was present.

Understanding how tendon precursor cells tension the extracellular matrix by Professor Karl Kadler (University of Manchester)

For the last presentation of the session, **Professor Kadler** explained how tendon precursor cells produce and regulate the tension, stiffness and elasticity of the collagen-rich matrix. Speaking on the incidence of collagen related medical conditions, it is evident not enough is known on the organisation and mechanical properties of collagen. How do cells create these parallel collagen fibrils which lead to the formation of such a strong tissue as tendon? Using horseradish peroxidase flooding, his lab have shown that collagen fibrils are aligned using fibripositors: these pull on fibrils using force generated by non-muscle myosin II. Studies in other labs as well as their own have created 3D culture models for tendon development which perform very well creating the tendon trademark crimp morphology. This has revealed a process called "hand-over-hand" in which an absence of cell migration creates the necessary tension in the tissue. Fibroblasts attach and pull collagen fibres using force generated non-muscle myosin II. In conclusion, Professor Kadler's research has found evidence of cell-dependent and matrix-dependent pathways to tissue tensioning, which could involve the proteinase membrane type 1 (MT1)-matrix metalloproteinase (MMP).

Session Four - BSMB Open Session

Chaired by Professor Tim Hardingham (University of Manchester) and Dr Che Connon (University of Reading)

Building complex tissue by Professor Clemens A. Van Blitterswijk (University of Twente, The Netherlands)

Professor Clemens A. Van Blitterswijk (University of Twente, The Netherlands) opened the BSMB Open Session by giving a talk entitled 'Building complex tissue'. He started his talk by illustrating how complicated tissue structure is and summarized the achievements of understanding this 'complex issue' in the last decade, using the example of 'biological effects of ions (Sr^{2+} , Cu^{2+} , Ca^{2+} , F^{2-}) for tissue instruction'. Clinical trials data showed that bone tissue engineering can have huge individual variation.

Professor Blitterswijk presented the results of fully synthetic implants based on calcium phosphate ceramic, with varying physicochemical and structural characteristics. They were at least equally successful as autografts and rhBMP-2 treatment in the management of a critical-sized bone defects. The data showed that the ability of ceramics to instruct cell and tissue development can be controlled merely by changing either the chemical composition or structural properties.

Surface topography has been widely recognised as a parameter to endow materials with bio-active surfaces. Professor Blitterswijk introduced a high-throughput screening platform for bio-active surface topographies 'TopoChip' which converged high content bio-imaging technology, typically used for screening of biologically active small molecules with micro- and nano-imprint technologies.

Finally, Professor Blitterswijk described a novel thin-walled chip-type micro-device formed by "microthermoforming" technology. These 3D micro-wells have been used in engineering artificial cellular microenvironments or niches in film-based multiwall assays, which made it possible to design and assemble structures prone to adequate tissue remodeling, predict and manipulate those developmental mechanisms in vitro, thus create more complex tissue in the future.

Intra-articular AMPA/kainate glutamate receptor antagonists alleviate inflammation, pain and pathology in rat antigen induced arthritis by Dr Cleo Bonnet (Cardiff University)

The second presentation was given by **Dr. Cleo Bonnet** (Cardiff University) on the topic of 'Intra-articular AMPA/kainate glutamate receptor antagonists alleviate inflammation, pain and pathology in rat antigen induced arthritis'. Concentrations of the neurotransmitter glutamate are greatly increased in synovial fluids of RA and OA patients. Dr. Bonnet described his study investigating whether the specific glutamate receptor subunits expressed in the synovium of patients with arthritis which mediate proinflammatory, degradative and proliferative responses could be therapeutically targeted to reduce disease progression and pain. By using the mono-articular antigen induced arthritis (AIA) rat model, the team used intra-articular injection of NBQX to inhibit AMPA/kainate receptors at the time of arthritis induction, prior to peak IL-6 levels. Over a 21 day period, lowered knee swelling with less pain related behaviour was found in NBQX treated rats compared to AIA rats. Ionotropic and metabotropic GluRs mRNA were differentially expressed in cartilage, synovium, meniscus, fat pad, patella, femoral head and shaft of rat knees. The results showed intra-articular NBQX treatment can alleviate inflammation, pain and pathology in arthritis in vivo and that GluRs are differentially expressed in knee joint tissues, thus supporting the hypothesis that kainate GluRs may be specifically targeted to ease pain, inflammation and pathology in arthritis.

Changes in collagen cross-linking in human intervertebral disc: with advancing age and severe disc degeneration by Dr Ma'an Al-Abbasi (University of Bristol)

The third presentation was given by Dr. **Ma'an Al-Abbasi** (University of Bristol) entitled 'Changes in collagen cross-linking in human intervertebral disc: with advancing age and severe disc degeneration'. His study was designed to investigate the changes and differences in cross-links (Hydroxylysyl-Pyridinoline (HL-Pyr), Hydroxylysinonorleucine (HLNL) and Pentosidine) associated with ageing and pathological degeneration in the same disc of each individual. Intervertebral discs were obtained from seven individuals with ages ranging from 63-90 years. The data showed a decrease in mature cross-link (HL-Pyr) and an increase in both intermediate (HLNL) and pentosidine cross-link levels with advancing disc degeneration when compared with non-degenerate regions of the same discs. This may represent an increase in matrix turnover. Other results showed mature and intermediate cross-links decline across the disc in both non-and degenerated regions with advancing age. Pentosidine showed little difference across the discs, but did show the expected age-related increase. Dr. Al-Abbasi indicated that the increase in pentosidine may be due to the resistance of glycated collagen to enzymatic degradation and therefore continues to accumulate with age and is present in greater proportion as the remaining collagen is lost. He hypothesised that the newly deposited collagen is deficient and that the residual pentosidine levels further contribute to the deterioration of disc integrity and function by increasing its stiffness and reducing elasticity. All this could have a profound deteriorating effect on the tissue matrix, leading to a mechanically less stable disc.

Equine mesenchymal stem cells lose their angiogenic properties when differentiated toward chondrogenic and osteogenic lineages by Miss Jennifer Bara (Keele University)

The fourth talk was given by **Ms. Jennifer Bara** (Keele University) entitled 'Equine mesenchymal stem cells lose their angiogenic properties when differentiated toward chondrogenic and osteogenic lineages.' A major challenge of osteochondral tissue engineering is to promote vascularisation of bone and prevent vascularisation of cartilage. Ms. Bara presented her study on the angiogenic/anti-angiogenic properties of equine bone marrow derived mesenchymal stem cells (eBMSCs) before and after chondrogenic and osteogenic differentiation *in vitro*. The data showed the production of angiogenic/angiostatic factors by eBMSCs decreased when differentiated into chondrogenic and osteogenic pellet. Endothelial cell tube formation significantly decreased when treated with conditioned media from chondrogenic and osteogenic cultures of eBMSCs, but in contrast, endothelial tubule formation was promoted by conditioned media from monolayer cultures of eBMSCs. The results demonstrated that eBMSCs are able to support angiogenesis *in vitro* and produce an array of angiogenesis related proteins. Chondrogenically and osteogenically differentiated eBMSCs can produce soluble factors that inhibit angiogenesis *in vitro*. Ms. Bara pointed out the balance of angiogenic factor production in differentiated cells may be of concern for osteochondral tissue engineering. Endothelial cell viability/proliferation assays should be used to investigate the mechanism of inhibition by differentiated cells in future.

Influence of small proteoglycans on nerve growth in the intervertebral disc by Mr Philip Jones (Keele University)

The fifth talk was given by **Mr. Philip Jones** (Keele University) entitled 'Influence of small proteoglycans on nerve growth in the intervertebral disc'. The study was to investigate the possible role of decorin, a small proteoglycan, in nerve growth regulation, as well as the growth factor, TGF-beta1, which binds to decorin. By using chick dorsal root ganglions as a model of nerve growth, the data showed that strips of decorin inhibited the growth of neurites in a dose dependant manner between 10 and 500ug/ml. Mr Jones also reported that decorin inhibition is reversed when treated with chondroitinase ABC but not AC, and neurite growth is not significantly affected by TGF-β1 either solely or in conjunction with decorin. This suggests that dermatan sulphate could play a role in the regulation of nerve growth by decorin. TGF-beta1 had no effect on neurite growth using this model system.

Beta-xylosides inhibition of chondroitin sulphate substitution on matrix proteoglycans perturbs the differentiation of bone marrow stem cells into a chondrogenic lineage by Dr Siyuan Li (Cardiff University)

The final talk of this session was given by **Dr. Siyuan Li** (Cardiff University) entitled 'Beta-xylosides inhibition of chondroitin sulphate substitution on matrix proteoglycans perturbs the differentiation of bone marrow stem cells into a chondrogenic lineage'. Chondroitin sulphate (CS) sulphation motifs on cell-associated proteoglycans (PGs) have been shown to be putative biomarkers of progenitor/stem cell sub-populations. They are supposed to play important roles in stem cell differentiation during development as CSs are also found in putative stem/progenitor cell niches at sites of incipient articular cartilage and other musculoskeletal tissues. Dr. Li outlined the study which investigated the importance of CS in the differentiation of bone marrow stem cells to the chondrogenic phenotype *in vitro* using p-nitrophenyl xyloside (PNPX) as a competitive inhibitor of CS substitution on matrix PGs. DMMB assay result showed an apparent delay in the cell bead formation in the BMSCs cultured with PNPX, indicative of the delay of chondrogenesis. Moreover, PNPX significantly inhibited/delayed the expression of chondrogenic markers including aggrecan, SOX-9 & type II collagen gene and/or protein expression. Furthermore, IHC analyses showed a decreased expression of native CS sulphation epitopes in chondrogenic media + PNPX. Dr. Li concluded by

highlighting the importance of CSs' role in allowing the chondrogenic differentiation to occur. The precise mechanism is still unclear, but CS sulphation motifs may be involved in the growth factor presentation needed for cell differentiation that leads to cell aggregation and extracellular matrix-cell interactions during chondrogenesis.

Session Five - Skeletal and cardiac muscle

Chaired by Dr Philippa Hulley (University of Oxford) and Dr Sarah Howat (King's College London)

Building matrix based solutions for disease: an update by Professor Doris Taylor (University of Minnesota, USA)

Professor Doris Taylor (University of Minnesota, USA) opened this session with a Skype presentation entitled 'Building matrix based solutions for disease: an update'. She began with a review of current cell therapy along the progression of ischemic heart disease (IHD) and pointed out that one hope of regenerative medicine is to treat underlying tissue damage at the level of the injury, rather than simply mitigating the effects of damage. Decellularization of donor organs such as heart, liver, and lung can provide an acellular, naturally occurring three dimensional biologic scaffold material that provides perfusion and even biologic structural cues that can drive cell behaviour.

Complex interplay between cells, their microenvironments and the vascular network are all critical drivers of myocyte physiology and function. Professor Taylor's group and their collaborators evaluated these in the decellularized organ constructs to explore new opportunities to dissect true potential for repair. Firstly, Professor Taylor's group showed it is possible to rebuild/recell a vascular network with membrane ECs, in both small and large diameter vessels and endocardium, with the function of clot formation inhibition and eNOS expression.

Then, she discussed the impact of matrix on stem or progenitor cell alignment, differentiation, function, and physiology as well as its use as an *in vitro* test bed to evaluate stem cell repair. Her team found *in vivo* DECELL matrix patches may partially prevent functional decline in a fractured heart. Another experiment showed cardiac derived PCs changed morphology and gene expression profiles based on matrix source. Further evaluation of the matrix architecture versus composition and organ source on stem cell commitment, differentiation and maturation are still underway.

Finally, Professor Taylor concluded that whole-organ tissue engineering is a potential breakthrough in vascularised tissue engineering, with an ability to leapfrog the current approaches with synthetic biomaterial and their associated obstacles. However, it is important not to claim victory prematurely and create overoptimistic expectations until indisputable success in animal models with organ failure is demonstrated.

A new methodological sequence to expand and transdifferentiate *in vitro* human cord blood derived CD133+ cells into cells with a cardiomyocyte-like phenotype by Dr Yuxin Cui (University of Bristol)

The second talk of this session was given by **Dr. Yuxin Cui** (University of Bristol) entitled 'A new methodological sequence to expand and transdifferentiate *in vitro* human cord blood derived CD133+ cells into cells with a cardiomyocyte-like phenotype'. Transplantation of antigenic-separated stem cells for human cardiovascular diseases such as myocardial infarction needs to be supported by experimental studies that allow refinement of the procedure. Dr. Cui reviewed his work on the optimization of a method for the expansion and subsequent differentiation of UCB derived CD133+ stem cells into a cardiomyocyte-like lineage. Immunomagnetic separated CD133+ cells were expanded and differentiated in a novel culture medium recipe that involves sequential signalling

factors. Expanded UCB CD133+ cells showed a cardiomyocyte-like phenotype following differentiation in vitro through expressing intracellular cardiac specific markers including cardiac-specific alpha-actin, myosin heavy chain and troponin I. These changes in phenotype are associated with the expression of cardiac-specific transcription factors Gata-4 and MEF2C. In addition, the changes in phenotype were associated with an upregulation of nuclear receptor transcription factors including peroxisome proliferator-activated receptor (PPAR-alpha), PPAR-gamma and retinoid X receptor. Based on the data, Dr. Cui indicated that it is possible to derive cardiomyogenic-like cells from UCB CD133+ stem cells. Further studies should be focused on functional acquisition and cytokine secretion of the differentiated cells. This will permit a more robust manipulation of these cells towards better engraftment and repair in patients with myocardial infarction.

Notch signalling in human induced pluripotent stem cells by Mr Stirling Yiin (University of Bristol)

Mr. Stirling Yiin (University of Bristol) then gave a short talk entitled 'Notch Signalling in human induced pluripotent stem cells'. In human embryonic stem cells (hESCs), Notch signalling has been demonstrated to be required for the formation of the three primitive germ layers and inhibition of Notch signalling resulted in a maintenance of pluripotency. Mr. Yiin presented his study of this pathway conducted on human induced pluripotent stem cells (iPSCs). The human iPSC colonies and embryoid bodies (EBs) were analysed for the expression of pluripotency genes, germ layer markers and Notch signalling genes, in the presence or absence of N-[N-(3,5Difluorophenacetyl) -L-alanyl]-S-phenylglycine t-butyl ester (DAPT), a gamma-secretase inhibitor. The data demonstrated the presence of Notch variants, its ligands and effector downstream genes in human iPSCs. The expression of these genes increased in EBs, suggesting a role for Notch during differentiation. The inhibition of Notch effector genes in EBs by DAPT led to significant downregulation of all differentiation markers. Mr. Yiin indicated that Notch inhibition is fundamental for the maintenance of iPSC pluripotency and plays a major role during their differentiation.

Stem cells and skeletal muscle regeneration by Dr Jennifer Morgan (UCL Institute of Child Health, London)

The final talk of this session was given by **Dr. Jennifer Morgan** (UCL Institute of Child Health) entitled 'Stem cells and skeletal muscle regeneration'. Firstly, Dr. Morgan updated the concept of stem cell with a highlight on satellite cells - the stem cells within skeletal muscle, which mediate the skeletal muscle repair, maintenance and regeneration. These cells may become activated in response to muscle injury and proliferate to create a pool of muscle precursor cells that express myogenic regulatory factors and differentiate into postmitotic multinucleated muscle fibres.

In vitro and in vivo studies showed that satellite cells are able to regenerate skeletal muscle and functionally reconstitute the satellite cell pool. Normal satellite cells grafted into muscles of the dystrophin-deficient mdx mouse, a model for Duchene muscular dystrophy, undergo little regeneration or self-renewal. But in the prior irradiated host muscle group, donor satellite cells contribute to significantly more muscle fibres. Irradiation incapacitates host satellite cells, but has no obvious effect on either the muscle fibres themselves, or the extracellular matrix. If the host muscle is injured by chemical or physical means that destroy the muscle architecture, donor satellite cell engraftment is not augmented. This result indicated the host muscle environment has a profound influence on satellite cell function.

Dr. Morgan indicated that satellite cells are a potential therapy to repair or replace muscle fibres that are lost as a result of ageing, or muscular dystrophies based on the results that young donor-derived satellite cells regenerate and self-renew equally well in young as in mature adult mdx nu/nu mice after pre-irradiation. But satellite cells are not systemically-deliverable and their capacity to regenerate skeletal muscle is reduced by even a short time in tissue culture. Another experiment had

showed that other postnatal stem cells, e.g. mesoangioblasts, pericytes, or CD133+ cells, may contribute to muscle regeneration after systemic delivery in animal models of muscular dystrophies. Finally, Dr. Morgan pointed out that the identity of the optimal muscle stem cell, which can be cultured without losing stem cell properties, delivered systemically and give rise to significant numbers of muscle fibres and satellite cells, still remains elusive.