

# British Society for Matrix Biology - Spring 2011 Meeting Report

written by Natasha Agabalyan,  
Matthew Mayhew and Henry Jia

## Advances in Musculoskeletal Repair and Regeneration

Organised by Wael Kafienah,  
School of Cellular & Molecular Medicine, University of Bristol

The Spring 2011 BSMB meeting was hosted by Bristol University on 11<sup>th</sup> and 12<sup>th</sup> of April and organised by Dr Wael Kafienah, with support from Jane Lohmann, Jan Cunningham and Prof Anthony Hollander. The meeting was held in the Wills Memorial Building. It was attended by ~130 delegates and comprised 5 sessions, each focused on a particular tissue in the musculoskeletal unit: bone, cartilage, tendons and ligaments and skeletal and cardiac muscle, with an additional Open Session. In addition to scientific presentations, Dr Michael Patnick, Head of Research and Education at Arthritis Research UK highlighted fellowship and funding opportunities. The prestigious IJEP-sponsored Fell-Muir Award was presented to Prof Bruce Caterson, Cardiff University for his work on Glycosaminoglycan Glycobiology.

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). It is vital to stress that the many bursaries provided to support attendance of this meeting were possible due the subscriptions paid by your BSMB membership and demonstrates our pledge to sustain and secure future matrix biology research into the future.

The meeting was generously sponsored by Thermo Scientific (Platinum), 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 1 - Bone**

Chaired by Prof Andy Pitsillides (Royal Veterinary College) and Dr Simon Tew (University of Liverpool), this opening session began with an exciting presentation by **Prof. Gordana Vunjack-Novakovic** (Columbia University, New York) entitled '*Craniofacial Tissue Engineering*'. She stressed the importance of tissue engineering, stating that as we live longer, we need spare parts and that bioengineering will help unlock the full regenerative potential of stem cells, to provide the dream of 'staying forever young'. Prof. Vunjack-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; relatively recent advances in medicine bumping up this figure only since the 20<sup>th</sup> Century. Prof. Vunjack-Novakovic went on to describe that cells respond to the entire context of their environment, for example, to cytokines and extracellular matrix (ECM), and thus we need to move on from *in vitro* to more physiologically relevant and controllable environments that more closely resemble those *in vivo*, aided by the use of various bioengineering solutions such as scaffolds and bioreactors. Current bioengineering is 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.

Alternative bioengineering solutions: 'naked' decellularised bone and hydroxyapatite-silk fibrin composites were presented. She described their potential to grow both bone and cartilage in one system, by interfacing a hydrogel containing chondrocytes on top of a mineralised scaffold with mesenchymal stem cells (hMSCs) which is under perfusion and then mechanically loaded. Gordana stated that they had obtained between 11-12% mineralisation by culturing hMSCs in the silk scaffold. She reported that this was relatively poor compared to normal bone, but does resemble very osteoporotic bone, and demonstrates the osteogenic ability of hydroxyapatite to differentiate hMSCs, from bone marrow or adipose tissue, to produce bone without the need of growth factors (GFs). Gordana stated that the rate of perfusion is also important, with the optimal perfusion rate ~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, Gordana demonstrated the ability of bioengineered scaffold tissues to heal flat bone and trabecular bone defects in a nude mouse and rat models respectively, significantly better than various controls.

Other advantages of these scaffolds were also emphasised. For example, some vascularisation has been observed using these bioengineered scaffolds. 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.

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 stated 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 3D constructs and patterns they had created and their relative efficacy in inducing differentiation and dedifferentiation gene markers. He highlighted the challenge of deciphering 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 interaction between various zones and constituents.

The third presentation was given by **Dr. Blandine Poulet** (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 highlighting osteoarthritis as a disease of multiple tissues, and 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 performed on the left (non-loaded) and right (loaded) knee joints with SCB thickness measured. 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.

The final talk in this session was by **Prof Molly Stevens** (Imperial College London) on '*New biomaterials strategies for orthopaedic tissue engineering*'. Prof. Stevens 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 hyaluronan (HA) containing liposomes which could slowly release Suramin to stop blood-vessel growth, creating an artificial hypoxic environment, resulted in cartilage growth. This demonstrated that simple biomaterials could be used for tissue engineering, and thus Molly posed the question regarding how complicated biomaterials should be, emphasising the need to strike a balance between the simplicity and organisation of the biomaterial and the GFs introduced. Prof. Stevens went on to discuss her lab's current work using bacterial  $\gamma$ -polyglutamic acid ( $\gamma$ -PGA) as a biomaterial with good mineralising potential. Esterifying the side-group could alter the water-solubility of the  $\gamma$ -PGA and she revealed that benzyl forms of esterified  $\gamma$ -PGA could increase the osteogenicity of hMSCs. She also showed how altering the mechanical properties (with Professor Paul Smith, ETH, Zurich) - by making a film of the polymer, aligning the fibres in the film - could result in an increase in modulus and tensile strength and allow for 'tailoring' of various 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, she 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 showed data related to the use of strontium ranelate. She outlined that this compound was already approved for human clinical use and that large doses had been shown to strengthen bones and reduce fracture incidence by stabilising osteoblasts and by preventing osteoclast mediated bone resorption. She showed how StronBone™ (strontium substituted bioactive glass) upregulated osteoblasts and downregulated osteoclasts. In a sheep femur model, use of StronBone resulted in stronger, stiffer bones with significantly less soft tissue. Prof. Stevens revealed that they have just received funding for a 68 patient trial, starting soon in the hospitals affiliated with Imperial College. Prof. Stevens finished by describing how they tracked differentiation and mineralisation over time in live cells via the use of Raman micro-spectroscopy. This provides in-depth analysis over the course of differentiation compared to traditional staining for mineralisation, which only detects the amount of mineral in fixed cells. Raman micro-spectroscopy can detect the level of crystallinity. Using this technique with traditional methods, the biomechanics of *in vitro* bone formation can be used to further elucidate and characterise cell-source-specific materials used to engineer bone.

## **Session 2 - Cartilage**

Chaired by Prof Anthony Hollander (Bristol) and Dr Emma Blain (Cardiff), the afternoon session opened with a 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 Prof Michael Buschmann (Montreal, Canada) unfortunately had to cancel his talk on '*Therapeutic technologies using natural polymers*' due to illness. Dr. Murphy began by highlighting the importance of AC hypoxia, at least in larger animals due to a lack of vasculature, stating that AC uses this hypoxic state to regulate cartilage-specific gene regulation. He went on to highlight the importance of AC in near-frictionless movement of load-bearing joints. Dr. Murphy stated that SOX9 is the main tissue-specific transcription factor that regulates these matrix genes for cartilage-specific function.

Dr. Murphy then elaborated on various factors that drive cartilage-specific gene expression, including mechanical loading, GFs such as TGF $\beta$  and BMP family members, and finally hypoxia. He showed that in human cartilage explants, a physiologically relevant oxygen tension (1-3% O<sub>2</sub>) upregulated COL2A1, Aggrecan and SOX9 mRNA, compared to non physiological oxygen tension (20% O<sub>2</sub>). He presented an overview of the Hypoxia-Inducible Factor (HIF) pathway and demonstrated via siRNA knockdown that HIF-2 $\alpha$  and not HIF-1 $\alpha$  was responsible for hypoxia-induced SOX9 upregulation, and in turn expression of type II collagen in isolated human chondrocytes. 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 PHD2 depletion enhanced HIF levels in both normoxic and hypoxic conditions and that this depletion of PHD2 upregulates SOX9, with greater upregulation at 1% O<sub>2</sub> 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 also inhibits IL-1 $\alpha$ -mediated aggrecan degradation. Chris 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 matrix metalloproteinases (MMPs) and aggrecanases, during hypoxia and showed that this upregulation of TIMP-3 is HIF-1 $\alpha$ -dependent and not HIF-2 $\alpha$ -dependent. Hypoxia was shown to downregulate MMP13, with inhibition of HIF-1 $\alpha$  upregulating MMP13. Dr. Murphy concluded his talk by reiterating that the anabolic effects of hypoxia are driven by HIF-2 $\alpha$ , which regulates SOX9 expression, and in turn cartilage-specific gene expression. Since both HIF-1 $\alpha$  and HIF-2 $\alpha$  are stabilised by inhibition of PHD2, PHD2 inhibition may represent a means of inducing both cartilage repair and protection in large animals. Murine cartilage may not be hypoxic because it is thinner - only a few cells thick - and although avascular may not be regulated in the same way as in larger animals.

The second presentation was given by PhD student **Steven Woods** (Newcastle) on '*miR324-5p in Osteoarthritis and Indian Hedgehog Signalling*'. He began his talk by giving an overview of microRNAs (miRNAs) and how they inhibit gene expression by targeting mRNA for degradation. He stated that due to imperfect base-pairing one gene can be targeted by many miRNAs. He went on to describe previous work in which murine Dicer, an important enzyme involved in the processing of miRNAs from their premature state, had been deleted in all cells expressing type II collagen, and how this resulted in high post-natal mortality and reduced skeletal size and enlarged hypertrophic regions. He also describe another paper in which miR140 deletion in mice lead to OA, but resulted in a far less severe phenotype compared to the conditional dicer knockout, indicating that other miRNAs may function in cartilage homeostasis. By screening miRNAs via RT-PCR Steven discovered miR324-5p upregulation in OA compared to healthy cartilage. He demonstrated how miR324-5p targeted 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 Lhh-stimulated C3H10T1/2 murine MSCs abrogated an increase of alkaline phosphatase levels, a marker of chondrocyte hypertrophy and bone formation, suggesting miR324-5p inhibits hypertrophy and bone formation via Lhh-Gli1 in OA.

To investigate other potential targets, a SILAC (stable isotope labelling with amino acids in cell culture) proteomics approach was taken. Thus, 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 Lhh pathway, an important pathway during OA, and that miR324-5p may also target various other genes.

The final presentation of the session was given by **Prof Susan Chubinskaya** (Rush University, Chicago) on '*BMP-7 in cartilage repair*'. Prof. Chubinskaya provided an overview of cartilage repair, stating that 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 family member in different types of human cell culture; overcoming the effect of catabolic cytokines in the presence and absence of serum. BMP-7 knockdown by siRNA was 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, and these were downregulated by siBMP-7. Interestingly, co-treatment with insulin-like growth factor (IGF-1) and BMP-7 provoked greater increases in proliferation and matrix deposition. 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 and 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. 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 included 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.

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,  $\alpha$  or  $\beta$  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 3 - Tendons and Ligaments**

Chaired by Prof Roger Smith (Royal Veterinary College) and Dr Hazel Screen (Queen Mary, London). the second day opened with a talk from **Prof Butler** (University of Cincinnati, USA) on normal matrix function and structure in 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), as well as their limited success (15-20% for the anterior cruciate ligament). The beginning of his talk explained the difference between different tissues and forces that are placed upon them during daily activities, stressing that ligament show a lower failure force than tendons. This was followed by a focus on the damage incurred by tendons and ligaments during ageing, which reduces biomechanical integrity (rabbit lose 25% of tendon strength between 1 and 4 years of age) and tendon fibril diameter; possibly due to the increased type V collagen (which regulates type I collagen). Ageing tendons are sensitive to loading, show reduced stiffness and a 50% reduction in thickness, area and compressive modulus. There is a substantial reduction in GAG content as well as a 200% increase in permeability. Professor Butler posed the following questions: Does tendon naturally heal? What are the patterns of gene and protein expression? Are cells migrating into wound sites? Using data from both rabbit and Col1-Col2 transgenic murine models, the talk was concluded with current work on improving natural healing with collagen-based matrices augmented with mesenchymal progenitor cells and mechanical stimulation in culture. Future work would focus on development, possible stem cell populations, growth control and how to achieve successful repair.

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

The third talk by **Dr Dudhia** (Royal Veterinary College) focused on the fate of mesenchymal stem cells in repair of tendon injuries of the equine forelimb. MSCs have already been used in this way but cell survival was found to be low 3-4 months after implantation. The aim of his work was to promote retention of MSCs in tendon after intra-lesional and intravenous injections. A clinical study of horses 21 days after onset was performed by randomised administration routes (regional perfusion, intravenous and intra-lesional). Although only 2.7-22.5% of cells remained labelled, this was sufficient 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. It was concluded that regional perfusion could be an alternative in the absence of core lesions.

For the last presentation of the session, **Prof Kadler** gave an excellent and engaging presentation highlighting new and exciting findings, decorated by some fascinating images. The precise content of this presentation has been withheld on the author's request.

#### **Session 4 - BSMB Open Session**

This was chaired by Prof Tim Hardingham (University of Manchester) and Dr Che Connon (Reading University). **Prof Clemens A. Van Blitterswijk** (University of Twente, Netherlands) opened the BSMB Open Session by giving a talk entitled 'Building complex tissue'. He started his talk by illustrating the complexity of tissue structure and summarised major achievements in understanding this 'complexity' in the last decade: using the example of 'biological effects of ions (Sr<sup>2+</sup>, Cu<sup>2+</sup>, Ca<sup>2+</sup>, F<sup>2-</sup>) for tissue instruction'. Clinical trials data showed that bone tissue engineering have huge individual variation. Prof Blitterswijk showed results of fully synthetic implants based on calcium phosphate ceramic with variable structural and physicochemical 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. Prof Blitterswijk described a novel thin-walled 3D chip-type micro-device formed by "microthermoforming" technology, used in engineering artificial cellular microenvironments or niches in film-based multiwall assays, which made it possible to design and assemble structures prone to tissue remodelling, predict and manipulate those developmental mechanisms in vitro, thus create more complex tissue in the future.

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 glutamate are greatly increased in synovial fluids of RA and OA patients. Dr. Bonnet's study investigated whether the specific glutamate receptor subunits expressed in arthritic synovium could be therapeutically targeted to reduce disease progression and pain. By using the mono-articular antigen induced arthritis (AIA) 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, reduced swelling with pain-related behaviour was found in NBQX-treated rats. Metabo- and iono-tropic GluRs mRNA were differentially expressed in cartilage, synovium, meniscus, fat pad, patella, femoral head and shaft. The results showed intra-articular NBQX can alleviate inflammation, pain and pathology in arthritis *in vivo* and support the hypothesis that kainate GluRs may be specifically targeted to ease pain, inflammation and pathology in arthritis.

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 (hydroxylslyl-pyridinoline, HL-Pyr; hydroxylslysinonorleucine, HLNL and pentosidine) associated with ageing and pathological. Intervertebral discs were obtained from seven individuals aged 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. Data were presented to show that mature and intermediate cross-links decline in both non- and degenerating 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, thus accumulating with age and becoming enriched as the remaining collagen is lost. He hypothesised that the newly deposited collagen is deficient and that the residual pentosidine levels further contribute to loss of disc integrity and function by increasing its stiffness and reducing elasticity, leading to a mechanically less stable disc.

The fourth talk by **Ms. Jennifer Bara** (Keele University) entitled 'Equine mesenchymal stem cells lose their angiogenic properties when differentiated toward chondrogenic and osteogenic lineages', highlighted a major challenge in osteochondral tissue engineering 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 production of angiogenic/angiostatic factors by eBMSCs decreased with chondrogenic and osteogenic differentiation. Endothelial cell tube formation significantly decreased when treated with conditioned media from chondrogenic and osteogenic eBMSC cultures, but in contrast, was promoted by conditioned media from monolayer eBMSC cultures. This suggests that eBMSCs can support angiogenesis *in vitro* and produce an array of angiogenic proteins. Chondrogenic and osteogenic 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.

The fifth talk was given by **Mr. Philip Jones** (Keele University) entitled 'Influence of small proteoglycans on nerve growth in the intervertebral disc'. This examined the roles TGF- $\beta$ 1 and decorin, a small proteoglycan, in nerve growth regulation. By using chick dorsal root ganglions as a model, the data showed that strips of decorin dose-dependantly inhibited

neurite growth (10-500ug/ml). Philip reported decorin inhibition was reversed by chondroitinase ABC treatment, but not AC, and neurite growth was not significantly affected by TGF- $\beta$ 1 either solely or in conjunction with decorin. This suggests that dermatan sulphate plays a role in the regulation of nerve growth by decorin. TGF-beta1 had no effect on neurite growth using this model system.

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 in 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 investigations into the importance of CS in bone marrow stem cell differentiation into chondrogenic phenotype using p-nitrophenyl xyloside (PNPX) as a competitive inhibitor of CS substitution on matrix PGs. DMMB assay showed an apparent delay in cell bead formation in BMSCs cultured with PNPX, indicating a delay in chondrogenesis. Moreover, PNPX significantly inhibited/ delayed expression of chondrogenic markers including aggrecan, SOX-9 & type II collagen gene and/or protein expression. Further, IHC analyses showed decreased native CS sulphation epitope expression the presence of 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 5 - Skeletal and cardiac muscle**

In this session chaired by Dr Philippa Hulley (University of Oxford) and Dr Sarah Howat (King's College London), **Prof Doris Taylor** (University of Minnesota, USA) opened 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 a naturally occurring 3-dimensional biologic scaffold material that provides perfusion and 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. Prof Taylor's group and their collaborators evaluated these in the decellularized organ constructs to explore new opportunities to dissect true potential for repair. Firstly, Prof 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 fractured hearts. She also showed that cardiac derived PCs changed morphology and gene expression profiles based on matrix source. Evaluation of the matrix architecture, composition and source on stem cell commitment, differentiation and maturation are still underway. Prof 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.

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'. Stem cell transplantation 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 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 expression of cardiac-specific transcription factors Gata-4 and MEF2C. In addition, changes in phenotype were associated with upregulation of nuclear receptor transcription factors including peroxisome proliferator-activated receptor (PPAR-alpha), PPAR-gamma and retinoid X receptor. 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.

**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, Notch signalling is required for the formation of the three primitive germ layers and inhibition of Notch results 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, Notch signalling and germ layer markers genes, in the presence or absence of a gamma-secretase inhibitor (DAPT). The data demonstrated the presence of Notch variants, its ligands and effector downstream genes in human iPSCs. 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 downregulation of all differentiation markers, indicating that Notch inhibition maintains iPSC pluripotency and their differentiation.

The final talk of this session was given by **Dr. Jennifer Morgan** (UCL Institute of Child Health) entitled 'Stem cells and skeletal muscle regeneration'. 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. pericytes, or CD33+ 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.

## **Poster prize winners**

**Mr Siyuan Li**, "Beta-xylosides inhibition of chondroitin sulphate substitution on matrix proteoglycans pertubs the differentiation of bone marrow stem cells into a chondrogenic lineage". Connective Tissue Biology Laboratories, Cardiff University, Cardiff CF10 3AX. E-mail: [lis8@cf.ac.uk](mailto:lis8@cf.ac.uk)

**Ms Sian Morgan**, "Extracellular matrix alterations in wounded mice corneas". Structural Biophysics Group, Cardiff University, Cardiff CF24 4LU. Email: [morgansr3@cardiff.ac.uk](mailto:morgansr3@cardiff.ac.uk)

**Mr Steven Woods**, "miR-324-5p in osteoarthritis and indian hedgehog signalling". Musculoskeletal Research Group, Newcastle University, Newcastle upon Tyne NE2 4HH. Email: [Steven.woods@ncl.ac.uk](mailto:Steven.woods@ncl.ac.uk)