BSMB Autumn 2007 Meeting Report, Keele

Shaping and Sensing the Extracellular Matrix.

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The autumn meeting of the BSMB was held at The Medical School, University of Keele on the 3rd and 4th of September. The meeting focused on two topics relating to the extracellular matrix (ECM); shaping and sensing. The meeting was organised by Dr Nicola Kuiper, Dr John Wardale and Professor Alicia El Haj. It attracted 100 participants. Financial support was provided by Smith & Nephew, Astrazeneca, DEVRO and The Company of Biologists. Trade exhibits were displayed by StarLab, Applied Biosystems, Corning Life Sciences and BOSE.

Professor David Lee (Queen Mary, University of London) opened the first session which focused on how the physical environment influences the shape of the ECM. He described a series of 3-dimensional studies using isolated chondrocytes grown in agarose. He used a mechanical testing rig which allowed physiologically relevant compressive loads to be applied to the chondrocytes. Under load, the cell deformation changed with compression but the nuclear deformation was variable. In addition, the chondrocytes had fewer cell processes and surface undulations when they were compressed. By combining this culture system with live cell imaging he demonstrated that the strain across chondrocytes under compressive load resulted in the deflection of cellular components, flexion of the cell membrane and possibly reduced cell volume. This was, however, not homogeneous. More recent work has shown that the application of compressive loads to chondrocytes attenuated their responses to the cytokine IL-1β and that this was mediated in part by MAP Kinases.

The next speaker, Miss Rebecca Harrison (University of Cardiff), described differences in the cytoskeleton from normal and osteoarthritic chondrocytes. Experiments were undertaken where the cytoskeletal structures of chondrocytes human OA and normal articular cartilage were examined and compared. Western blots examined the expression of the three filament types but showed no difference between OA and normal samples. Real-time PCR revealed an increase in tubulin and thymosin B4 in OA chondrocytes. Confocal microscopy showed that in normal chondrocytes, all three types of filament were uniformly distributed, whereas OA chondrocytes displayed uneven distribution, with actin being confined to the cell periphery, tubulin appearing to be disrupted, and vimentin presenting loose and diffuse staining. Disruption of the cytoskeleton was also carried out on both normal and OA cells. This led to changes in gene expression. Whilst changes in expression of inflammatory cytokines, MMP-14 and aggrecanase were similar in both sample types, some genes changes, such as SOX9, IGF1 and TGFβ1 differed between cells. Collectively these results would suggest that cytoskeletal changes are involved in cytoskeletal changes in OA chondrocytes, and would also indicate that distorted cytoskeletal arrangement results in the altered phenotype seen in OA chondrocytes rather than vice-versa.

Miss Blandine Poulet (The Royal Veterinary College, London) continued the session with an investigation into *in vivo* loading of murine knee joints as a model for OA. This investigation developed a non-surgical murine model of joint loading, to determine the mechanisms underlying the development of OA. The right knee of male and female Str/ort and normal (C57BL/6) mice were cyclically loaded, 3

times a week for 2 weeks with 9.5 and 9N forces (0.1Hz, 10sec/cycle for ~40cycles), which should be sufficient to promote changes in bone. Histological staining with Toluidine blue, visualised via light microscopy, allowed the OA-like lesions to be graded (0-6) in the medial/lateral and tibial/femoral compartments. Grade 6 represented a situation where less than 80% of the chondral surface remained. Results suggested that normal mice develop OA-like lesions in response to 2 weeks of controlled loading. The Str/ort mice developed bilateral symmetrical lesions with greater severity. The normal loaded mice only developed lateral femur lesions. This was found to be a promising model of OA, with more homogeneous, synchronised and faster onset than spontaneous models.

The session was concluded by Dr Jim Ralphs (University of Cardiff) who discussed cell-cell interactions in tendons and how this might control collagen deposition. He showed that tenocytes *in situ* were arranged longitudinally with many lateral cell processes and membranes surrounding bundles of collagen fibres. He showed that these cell processes contained the gap junction connexin 43 but not connexin 32. However both these molecules were present at longitudinal connections between the cells. General inhibition of all gap junctions in tendon cell cultures resulted in a down regulation of collagen synthesis, as did antisense knockdown of connexin 32. However antisense knockdown of connexin 43 resulted in increased collagen synthesis indicating an important role for connexins in the lateral cell processes in controlling collagen secretion.

After a break for refreshments the first ever recipient of the BSMB Young Investigator Award afternoon's Young Investigator Award, Dr Julie Huxley-Jones (University of Manchester), gave an eloquent presentation on the evolution of the ECM. She had performed complex comparisons between the human genome and that of the invertebrate *Ciona Intestinalis.* She explained that the development of the full spectrum of human collagen genes occurred as a result of two genome duplications. She described how ECM genes are overrepresented in the human genome, compared to more primitive species. By exploiting general descriptive tags assigned to all genes she has been able to show that other classes of genes are over represented in the human genome. These include genes involved in development, adhesion signalling and differentiation. She argued that these sorts of genes are what make us human, and so extracellular matrix genes are important for defining us.

Following this Professor Brian Johnstone (Oregon Health & Science University, USA) opened the second session which focussed on the relationship between tissue regeneration and the shape of the ECM. He spoke about the difficulties of repairing articular cartilage. He presented his work on mesenchymal stem cells (MSCs), which can be differentiated into chondrocytes if cultured as cell pellets in a defined medium. He described the problems of scaling up the system. This included the serious issue of terminal differentiation of the chondrocytes. To improve the scalability of MSC derived cartilage grafts he described his work using a photopolymerisable hydrogel. The MSCs differentiate well within the hydrogel and the process has been improved through the optimisation of the cell seeding density and the incorporation of high molecular weight polyethylene glycol into the gels. Exposure to chondrogenic factors was investigated to see if brief exposure to TGFβ1 increased the chondrogenesis of periosteal stem cells. It was found that monolayer pre-treatment with medium containing TGFβ1 resulted in decreased matrix production. TGFβ1 withdrawal increased glycosaminoglycan production but it decreased the collagen content of the neocartilage. Dexamethasone withdrawal

was also investigated, and results indicated that its presence is necessary for 5 days, but if it is withdrawn at 7 days GAG production increases and collagen production decreases. Parathyroid hormone-related peptide (PTHrP) was also introduced in an attempt to prevent hypertrophy. Early findings implied that treatment for 1-3 weeks did not have an effect on hypertrophy nor did it prevent the synthesis of type X collagen. PTHrP treatment did, however, slow the appearance of type X collagen.

Mr Etienne O'Brien (University of Manchester) discussed the use of MSCs in the repair of tendon injuries. He described how he has developed a mouse model of tendon injury created using a 23 gauge needle. This resulted in mineralisation of tissue close to the wound edge. In some cases the tissue in the contra lateral tendon mineralised too. This model system will soon be used to examine the usefulness of MSCs delivered in a fibrin gel as a cell-based tendon repair treatment.

Dr Sophie Gilbert (University of Cardiff) explained that cell death occured close to articular cartilage lesions and there was little or no migration of the cells from healthy cartilage into a wound site. She showed that cell death could be reduced by the application of either a caspase inhibitor or necrostatin 1. Glycosaminoglycan loss at the cartilage wound sites was also reduced by the application of these inhibitors. Encouragingly, the caspase inhibitor was also able to promote integration of two aligned cartilage wound edges.

The session and the day were drawn to a close by Professor Tim Hardingham (University of Manchester) who gave an enthusiastic overview of the current paradigms in tissue engineering, giving four specific examples. The first example was that of bladder regeneration. The approach was pioneered by Dr Tony Atala in Boston, USA. It was an example of an expensive, clinically driven procedure to treat a special set of needs. The second example was an off the shelf, xenogenic product. This was the work of Dr Steve Badylak in Pennsylvania, USA. It illustrated how a temporary scaffold could enhance a patient's own repair mechanisms. He used porcine small intestinal submucosa as a biomaterial. The third example was from Tim's laboratory. Dr Alan Murdoch used a MSCs pellet culture system which illustrated the importance of the delivery and context of signals for tissue regeneration. Finally the fourth example described the replacement of a complex organ function such as the kidney. He described the pioneering work of Dr Marc Clancy and Dr Paul Brenchley who used embryonic kidney rudiments as a form of kidney transplantation.

The second day of the conference focused on sensing the ECM. The opening session featured Dr Anna Plaas (RUSH, Chicago, USA) who described her work on the expression of the Toll-like receptor-4 (TLR4) in murine knee joints. TLR4 is a candidate for the inflammatory response to cartilage repair. Anna described an *in vitro* model utilising fresh human tissue and compressive injury. She investigated the expression of TLR4. Results found that TLR4 was constitutively expressed in the tissue. She then used an *in vivo* non-surgical murine OA model to study TLR4 in more detail. TGF-ß injections caused cartilage degeneration and HA injections prevented cartilage degeneration. Results suggested that there is an inflammatory component to cartilage repair in the overuse-OA model and that HA injections may offer a potential therapy.

The next speaker, Miss Jenny Lindop (University of Newcastle), described her investigations of superoxide dismutases (SOD) gene expression in OA cartilage. Expression of SODs, particularly SOD2, is significantly down-regulated in OA cartilage. Epigenetic modulation via promoter methylation of SOD2 was observed in

cancer cell. This process is altered with increasing age and disease, and therefore may act as a regulatory mechanism, resulting in the changes seen in OA cartilage. Immunohistochemical staining for SOD2 showed down-regulation of expression in OA tissue. Treatment of OA chondrocytes with 5'-aza-2-deoxycytidine significantly increased SOD2 expression. This suggests that DNA methylation could be a novel-therapeutic target for OA.

Professor Sally Roberts (The Robert Jones & Agnes Hunt Orthopaedic Hospital/ University of Keele) followed with a presentation of a study to investigate the distribution of lubricin (PRG4) in biopsies of autologous chondrocyte implantation tissue. Lubricin is a glycoprotein with a mucin-like domain; that undergoes high levels of post-translational modification. Biopsies were taken from 36 patients, averaging 18 months post operative and 36 years of age. Immunohistochemistry revealed lubricin in the underlying bone in all samples where bone was present and in the cartilage (32/36). 26 patients showed lubricin localised to the cartilage surface in the ECM or within the cells themselves. 7 samples showed no staining in the surface layer with 3 unclear and another 25 samples showed staining towards the deep zone of the cartilage. The staining pattern seems typical of lubricin in cartilage which has been recreated in 70% of the repair tissue biopsies. The deeper zone presence however was unusual and may indicate a remodelling stage as more type II collagen is available there.

The morning session on biomarkers of disease ended with a talk by Dr Rose Maciewicz (Astrazeneca) who described where research and development meet in the translational activities of OA. She explained that the path for drug discovery involves a number of essential steps, including target identification, positive hits and leads, optimisation, normalisation, concept development, and the final launch. Overall drug development requires intelligent us of *in vitro*/*in vivo* models to reduce the risk of failure in human clinical trials. Using it for clues to direct and refine research, setting dose ranges, refining methodologies, and in the case of OA, to discover links between structural changes and functional outcomes.

After coffee there was a BSMB Society session which featured five talks on different aspects of matrix biology. Dr Simon Tew (University of Liverpool/University of Manchester) presented his work on osmotic regulation of SOX9 in human articular chondrocytes through p38 MAPK-mediated mRNA stabilisation. He described how fluctuations in osmolarity affect levels of SOX9 mRNA. Since these changes involve signalling through the p38 MAPK pathway this could be a mechanism in which osmolarity regulates ECM production. To demonstrate this, human articular chondrocytes were cultured in monolayer and subjected to a variety of osmotic concentrations with or without the addition of a ROCK inhibitor, which inhibits stress fibre formation, and a p38 MAPK inhibitor. Results indicated that an increase in medium osmolarity is associated with p38 MAPK activation and its subsequent stabilisation of SOX9 mRNA, therefore resulting in chondrocytes ECM synthesis.

Dr Aileen Crawford (University of Sheffield) discussed her research into cartilage tissue engineering using MSCs sourced from synovial fluid. Results showed that the constructs of synovial fluid-derived MSCs were large and more consistent in size than constructs of bone marrow-derived MScx. Synovial fluid-derived clones had a significantly higher growth rate, increased chondrogenesis and appeared to be more resistant to hypertrophy and ossification. These findings therefore suggest that synovial fluid may be a valuable alternative source of MSCs.

Dr Darren Plumb (University of Manchester) described how targeted type XXVII collagen mutations cause lung and skeletal phenotypes. Most vertebrate fibrillar

collagens can be divided into two related but distinct clades: the A clade, whose members share a homologous N-terminal von Willebrand factor type C domain, and the B clade, where collagens posses a thrombospondin domain. Type XXVII collagen, however, has an unusual molecular structure in that it has no minor triple helix and a short interrupted major triple helix; for these reasons it represents a new clade, type C.

Miss Siriporn Peansukmanee (University of Liverpool) described a study which compared ECM degradation and MMP levels following cytokine stimulation or changes in oxygen tension. Equine cartilage explants were cultured either in the presence or absence of human recombinant inflammatory cytokines TNFα, IL-1ß, OSM or IL-1ß + OSM, and at 20% or 1% oxygen tension for 5 days or 4 weeks. Results indicated that although equine proteoglycan is degraded by cytokines, only TNFα has an effect on collagen loss. Contrary to previous work which reported a positive effect of hypoxia on articular chondrocytes metabolism, this study did not show any effect of oxygen tension on ECM degradation.

Dr Emma Blain (University of Cardiff) talked about her investigations into the maintenance of chondrocyte phenotype by the vimentin intermediate filaments. Due to its concentration in areas of mechanical loads in cartilage, vimentin has been suggested as a mechanotransducer. It has also been associated with cytoskeletal changes in OA. Results indicated that vimentin filament integrity and arrangement is important in the maintenance and homeostasis of articular cartilage. Further work will examine whether inhibition of the ERK pathway restores the vimentin cytoskeleton.

The final session on sensing the ECM focussed on the sensation of pain. The first speaker, Professor Bruce Kidd (Queen Mary School of Medicine, London) provided an overview of why joints hurt; explaining that pain is not an inevitable consequence of OA and that there is no clear correlation between joint pain and arthritic disease severity. Joints seem to end up causing pain due to pain receptors becoming hypersensitive to chronic pain stimuli. He presented data which suggested that this be driven by alterations in nerve cell receptor expression- particularly of the TNFα receptor in arthritis, and of the NMDA receptor in spinal pain. Overall he argued that understanding the shift of the pain sensitisation curve is essential for understanding joint pain and underlined the need for drugs which return sensitisation to its normal levels rather than analgesics which lead to desensitisation.

The next speaker was Miss Elaine Garvican (University of Liverpool) who showed that activated protein C, also known as factor V in the clotting cascade, could synergize with IL-1β but not TNFα or oncostatin M to induce collagen breakdown in equine articular cartilage explant cultures. APC and Il-1β induced a maximal level of MMP gene expression and activity whilst a global MMP inhibitor prevented collagen release in the system. The application of corticosteroids such as dexamethasone to the explants prevented the APC/IL-1β induced collagen release.

Professor Tony Day (University of Manchester) then spoke about a new role for the TSG6 protein in bone remodeling. He showed that TSG6 could prevent bone erosion by osteoclasts in response to M-CSF and RANKL which would appear to be mediated through the binding of TSG6 to RANKL. This binding is interesting as it requires both the link and cub module of TSG6 for maximum affinity. Paradoxically, TSG6 knockout mice have increased bone density however. Further studies were able to show that TSG6 can potently inhibit BMP2 mediated osteoblastogenesis and binds BMP2 strongly. As for the RANKL interaction, maximum affinity is observed only

when both cub and link modules are present in the protein. Therefore, TSG6 appear to have regulatory roles in both bone resporption and deposition and presents an attractive target for osteoporosis and other bone diseases.

The final speaker was Dr Jason McDougall (University of Calgary, Canada) who returned to the pain theme to present data on joint pain in animal models. He showed by measuring joint nociceptor (acute pain receptor) spike activity in rats that abnormal movement caused firing of the nociceptor at increased frequency. He focused on the cannibinoid receptor CB1 and by using an agonist to this receptor was able to demonstrate reduce nociceptor firing in response after both normal and abnormal movement. A CB1 antagonist increased firing in response to normal movement but did not alter the firing rate after abnormal movement as he explained that these receptors were already firing at their maximal rate. He then used the same electrophysiological measuring system to examine nociceptor firing in both 2 month and 3 year old Dunkin Hartly guinea pigs. The old guinea pigs had osteoarthritis, the severity of which was determined by μCT scan. As expected, young animals increased nociceptor activity after abnormal rotation but old animals showed increased firing rates with even normal rotation. Importantly, principle components analysis revealed that in the old animals, the degree of nociceptor firing did not correlate with the severity of joint disease.