Contents

Your 2005 subscriptions are now due
Please see the editorial on page 2 for details

2 Editorial by Anthony Hollander

2 Current BSMB committee Contact Information

3 New committee members Call for nominations

3 New arrangements for bursaries Application details

3 Publication of meeting abstracts Details

4 Forthcoming BSMB meetings Meeting details for 2004-2006

4 XIXth FECTS meeting report by Vicki Anderson et al

16 BSMB autumn 2004 meeting report by Laura Sudre and Frank Cheung

20 Details and registration form for Spring 2005 meeting Please register NOW
Editorial

By Anthony Hollander

Welcome to the 65th edition of Connective Issues. Your membership subscription fee is now due. Members can pay by cheque if they prefer, however it is considerably easier for the committee if you pay by Standing Order. You can do this using the membership application form on our website: http://www.BSMB.AC.uk/FRAMES/GENERAL/Membership.htm

If you pay by Standing Order then please can you make sure that you are paying the correct subscription fee. You should only be paying the reduced fee of £2 if you are still a student. Otherwise you should be paying £10. We will conduct a follow-up audit in a few weeks time and anyone who has not paid their subscription will be notified that they are no longer a member.

We generally have rather few applications for bursaries to attend BSMB and FECTS conferences. For the most recent meeting in Bristol there was only one application, from Laure Sudre (Manchester), who received £125 towards her costs. In view of this rather poor level of uptake, the BSMB committee has asked Ray Boot-Handford to take on responsibility for updating and streamlining the procedures. He has put considerable effort into this and a summary of the new system can be seen elsewhere in this Newsletter as well as on our website.

Please can you make sure all young members are aware of this scheme and that they take full advantage of it.

Please can I urge all of you to register early and submit your abstracts for the Spring meeting in Liverpool (details are at the end of this Newsletter).

The XIXth FECTS meeting was held in Sicily in July 2004 and was a great success despite the exceedingly hot weather and variable air-conditioning! A scientific report of the meeting can be found below.

John Tarlton and I would like to thank all of you who supported our autumn meeting in Bristol. The scientific report can be seen later in this Newsletter. There were a total of 151 delegates, which included 49 full members, 30 student members, 65 non-members and 7 student non-members. The high number of non-members was presumably because the meeting was joint with TCES. There were 71 posters of which 30 were entered into the poster prize competition. Three winners were selected by delegate voting. Each delegate was able to cast a vote for up to 3 posters. A total of 180 votes were cast (ie 60 people with 3 votes each). The winners were Ana Macintosh (Sheffield) with 18 votes, Hadi Mirmalek-Sani (Southampton) with 17 votes and Amnda Hall (Cardiff) with 13 votes. The conference dinner on board SS Great Britain was memorable if only for Bruce’s epicurean style of after-dinner speaking.

Current BSMB Committee

Officers:
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Call for Nominations for two New Committee Members

Two committee members, John Tarlton and Malcolm Lyon, will have completed their term of office in April 2005. We are therefore seeking two new members to join the committee. If you are willing to get involved and perhaps to organize a meeting then please consider putting yourself forward. We are particular keen to receive nominations from members who come from regions or Universities/companies not already represented on the committee.

Deadline for Nominations: Friday 20th February 2005
If more than one nomination is received then an ELECTRONIC ballot will be organized

BSMB Conference Bursaries

The Society offers bursaries for younger researchers (e.g. PhD students and young postdocs) to attend its annual Spring and Autumn meetings. Due to the low numbers of applications received for recent meetings, the BSMB Committee has decided to reorganise the bursary scheme.

The BSMB meeting bursary scheme will in future (i.e. as of the Spring 2005 Liverpool meeting) have two distinct application routes.

Conference Presentation Bursaries
The BSMB will offer 4 ‘Conference Presentation’ bursaries per meeting for young researchers wishing to present their research at the Spring or Autumn Society conferences. The research topic must be relevant to the BSMB but need not be on the ‘theme’ of the meeting. Applications for a bursary must be accompanied by the abstract of the research to be presented.

The bursaries (of up to £125) cover all conference costs (e.g. registration, accommodation, conference dinner) and a contribution toward travel costs. In exceptional circumstances, a request for further assistance with travel costs may be considered by the committee. For more details and application procedure, see: http://www.bsmb.ac.uk/ under Bursaries

REVISED closing date for Liverpool (Spring 2005) meeting Conference Presentation Bursary applications: January 28th 2005 with decision by mid-February.

Conference Reporter Bursaries
The BSMB will offer 3 ‘Conference Reporter’ bursaries for each meeting. These bursaries are open to all members of the Society and applicants simply need to undertake to write a report on the talks at the conference for publication in the Society Newsletter ‘Connective Issues’ and subsequently in the International Journal of Experimental Pathology. (This is an excellent opportunity for individuals wishing to develop their scientific writing and reporting skills. If desired, reports on each talk can be attributed to the individual ‘reporter’ responsible enabling the resulting issue of IJEP and Connective Issues to be used by the authors as examples of their output, for instance within a Profession Skills Development Portfolio). Applicants need not be presenting a poster at the meeting. For more details and application procedure, see: http://www.bsmb.ac.uk/ under Bursaries

REVISED closing date for Liverpool (Spring 2005) meeting Conference Reporter Bursary applications: January 28th 2005 with decision by mid-February.

FECTS Bursaries
Bursaries for FECTS meetings will be run under the same general regulations as the Conference Presentation Bursary scheme described above but substantially larger awards will be available. The competition for FECTS bursaries will be announced approximately 6 months prior to each meeting.

Publication of Meeting Abstracts

A meeting report and abstracts for the Spring 2004 meeting in Manchester was published in the October 2004 edition of IJEP.

The report and abstracts for the Autumn 2004 meeting in Bristol will be published in IJEP shortly. The report can also be read elsewhere in this Newsletter.
Forthcoming BSMB Meetings

The spring 2005 meeting will take place on 21st/22nd March at the Sherrington Buildings, University of Liverpool, and is being organised by Dr Anne Vaughan-Thomas, Dr. Pete Clegg and Prof. Stuart Carter. Further details and the abstract and registration forms are at the end of this Newsletter.

The autumn 2005 Meeting is to be held at the University of Manchester on September 12th/13th. The theme will be “Pathobiology of Bone and Cartilage” and the meeting organizers are Dr. Mike Briggs and Dr. Ray Boot-Hanford. Invited speakers will include Frank Barry (Galway), John Bateman (Galway), Michael Briggs (Manchester), Peter Byers (Seattle), Kathy Cheah (Hong Kong), Bruce Caterson (Cardiff), Philippa Francis-West (London), Tim Hardingham (Manchester), Dick Heinegard (Lund), Frank Luyten (Leuven), Stefan Mundlos (Berlin), Checco Ramirez (New York), Gillian Wallis (Manchester) and Mat Warman (Cleveland).

Early warning of future meeting:
April 10th/11th 2006, Queen’s College Cambridge: Dr. Graham Riley and Professor Gillian Murphy: “Proteases: the cutting edge of cell biology”

Meeting Report:
XIXth FECTS Meeting, Taormina-Giardini Naxos, Italy 9-13th July 2004

By Vicki Anderson, Emma Blain, Philippa Callender, Ethne Comerford, Lindsay Davies, Rhiannon Fish, Sophie Flood, Magali LeGoff and Dimitrios Zeugolis.

Last Summer the Italian Society for Connective Tissue hosted the biannual meeting of the Federation of European Connective Tissues Societies in Taormina-Giardini Naxos. A welcome reception was held during the break in the opening Plenary Lecture Session for the 408 delegates who attended the meeting. The BSMB was well represented at the meeting with 89 delegates from the UK.

The registration fees covered 85% of the meeting expenses and external main sponsors of the Meeting were the Sicily Region Governor Office and the University of Messina. Minor sponsors were laboratory material suppliers: Bio Rad Laboratories Italy, Applied Biosystems and Celbio.

Friday 9th July - Plenary Session 1:
Chair: John E. Scott (Honorary Chairman of the Meeting)

After Alberto Calatroni opened the meeting, Renato Iozzo (Philadelphia, USA) presented recent work on regulation of angiogenesis by endorepellin, a fragment of perlecan. The structure of this proteoglycan was presented in the introduction and the talk then focussed on the C-terminal domain of perlecan called endorepellin and its action on angiogenesis. Endorepellin inhibited VEGF-induced endothelial cell migration in vitro and also inhibited VEGF and tumor-cell mediated angiogenesis in vivo using Matrigel plug and CAM assays. To investigate the mechanism, the effects of endorepellin and LG3 domain, a fragment of endorepellin, on endothelial cells were studied. They both affected capillary morphogenesis and reversibly disrupted actin stress fibers and focal adhesions. Endorepellin and the LG3 domain were shown to interact with the α2I domain of the α2β1 integrin, triggering an increase in intracellular cAMP levels and PKA and FAK activity. Transient activation of p38 MAPK and Hsp27 caused disassembly of actin stress fibers and endothelial cell cytoskeleton focal adhesion.

Anthony Hollander (Bristol, UK) discussed the current approaches for cartilage repair and the quality of the generated repair tissue. Surgical strategies, including microfracture and mosaicplasty, for the repair of defined cartilage lesions generated by traumatic injuries such as cruciate ligament damage or by osteochondrosis dessicans were evaluated. Autologous chondrocyte implantation was rated as successful, although the generated repair tissue is mostly fibrocartilaginous. The question of whether this was tissue regeneration was raised.

Professor Hollander went on to discuss work using expanded chondrocytes seeded onto a Hyalograft C scaffold, which had been implanted into cartilage defects. Analysis of glycosaminoglycan, hydroxyproline, type I and II
collagen content from biopsies at 8-24 months showed a equal mix of hyaline-like and fibrocartilaginous repair tissue. A short discussion followed regarding the SLRP content of tissue engineered (TE) cartilage. Decorin and fibromodulin levels were elevated compared to articular cartilage but it is the significantly increased levels of lumican that are of interest. It has been hypothesised that decreasing the levels of lumican may be a target for increasing the collagen content of TE cartilage.

The long term aim for cartilage repair is whether this cell therapy approach can be used in osteoarthritic (OA) patients. Can TE cartilage grow in OA sufferers or will it be inhibited by the arthritic process? Some evidence from patients with cartilage lesions who have developed secondary OA surprisingly show a higher level of type II collagen and proteoglycan content than patients with no arthritis. However there are still issues regarding cell source and ways of maintaining cells within unconfined lesions. The lecture was concluded with the encouraging knowledge that TE cartilage is a reality and that potentially cartilage repair would be possible for OA patients in the future.

After the welcome reception, **Israel Vlodavsky** (Haifa, Israel) introduced the enzyme heparanase and its role in cancer progression and normal development. Heparan sulphate proteoglycans are known to play an important role in self-assembly and barrier properties of basement membranes and other extracellular matrices. Degradation of heparan sulphate therefore affects tissue integrity and hence normal and pathological events involving cell migration and responses to changes in the extracellular microenvironment. Mammalian heparanase, an endo-[β]-d-glucuronidase, is synthesised as a latent 65KDa precursor that is processed at the N-terminus of the molecule revealing the active site to generate a highly active heterodimer comprising 50KDa and 8KDa subunits.

Preferential expression of heparanase is present in a number of tumour tissues and metastatic cell lines. In addition, increased expression of heparanase in bladder and pancreatic cancer patients correlates with a reduction in post-operative survival. Elevated levels of heparanase were detected in sera of metastatic tumour bearing animals and cancer patients and in the urine of some patients with aggressive metastatic disease. Phenotypic changes in transgenic mice that over-express heparanase include enhanced neovascularisation and disruption of the epithelial basement membrane within the mammary glands causing excessive branching and widening of ducts. These mice also exhibited an accelerated rate of hair growth and increased vascularisation. Collectively, these data indicate that heparanase plays an important role in matrix remodelling, neovascularisation, and tumour cell invasion, all critical processes in cancer progression.

Cleavage of heparan sulphate (HS) at every 8-10 sugar residues induces the release of active HS-bound growth factors that can potentiate receptor binding and a cellular response. Fibroblast growth factor (FGF) is one such chemokine that becomes presentable at the cell surface and may be responsible for downstream signalling events that allow cell proliferation, invasion and angiogenesis during tumour growth. Addition of latent heparanase to endothelial cells enhances Akt phosphorylation independent of enzymatic activity or the presence of HS proteoglycans. Latent heparanase also stimulated phosphatidylinositol 3-kinase-dependent endothelial cell migration and invasion. These results suggest for the first time that heparanase activates endothelial cells and elicits angiogenic responses directly. These effects appear to be mediated by a heparanase cell surface receptor but to date no such receptor has been identified.

Heparanase processing was shown to take place in the lysosome and uptake mediated by cell membrane HS proteoglycans. Site-directed mutagenesis experiments determined that Tyr157 was important for heparanase processing. Human heparanase is localised primarily to lysosomes and late endosomes surrounding the nucleus. Some heparanase has also been shown to be cell surface associated and secreted. A proportion of cytosolic active heparanase is translocated to the nuclei where it may degrade nuclear HS contributing to the regulation of gene expression and signal transduction. Expression of heparanase itself is tightly regulated by several mechanisms. Promoter methylation may modulate expression, as demethylating drugs induced upregulation of heparanase expression and activity in rat glioma cells which ordinarily lack heparanase activity. Oestrogen sensitive response elements within the heparanase gene promoter may provide a new molecular pathway in breast cancer cells through which oestrogen may induce heparanase over
expression and thus promote cell invasion and neovascularisation.

Recent results from heparanase gene silencing experiments indicate that down regulation of heparanase expression is effective at reducing experimental metastatic potential and tumour angiogenesis. This anti-cancerous effect of gene silencing and heparanase-inhibiting molecules, taken together with the identification of a predominant functional enzyme and its specific expression make it an ideal target for anti-cancer drug development.

Joan Marini (Bethesda, USA) discussed the effects of bisphosphonates on the bones of children and mice with osteogenesis imperfecta (OI). OI (also known as brittle bone disease) is a condition, which can result in fractures and vertebral compression in young animals associated with very mild trauma. The clinical classification of this condition grades the severity of the disease presentation from Type I-IV with I-being very mild and IV being lethal within the first year of life. Patients with this condition can present with a short stature, large cranium and a flail chest. The condition is caused by defects in collagen type I molecules due to mutations in the COL1A1 or COL1A2 genes.

Animal models used in the investigation of this condition have included the glycine substitution model (Gly349-Cys), and the Brittle (Brtl) Type IV OI mouse model. The Brtl mice have a smaller flattened cranium and demonstrate bowing of the long bones. Treatment, to date, for this condition has historically been oral bisphosphonates, which have been used, successfully in post-menopausal women, however there have been no controlled trials on the use of this drug to treat OI. Bisphosphonates are analogues of inorganic pyrophosphates, which are rapidly cleared by osteoclasts, thus inhibiting bone resorption seen in this condition. However, the drugs have no effect on the mutant collagen resulting in some poor quality bone.

Two controlled trials were performed in this study to examine the effect of alendronate (pharmaceutical grade bisphosphonate) in mice and pamidronate in children. In the murine study, four groups of Brtl and Wild type (wt) mice were used and divided into placebo and treatment groups; twenty mice were assigned to each group. The mice were treated from 2-14 weeks of age and the drug’s effects on the femurs and lumbar vertebrae examined by measuring bone mineral density (BMD), using micro-CT analysis, geometry, and mechanical properties. The BMD was measured separately for the femurs and spine and was increased in the treated groups of both wt and Brtl mice. The micro-CT analysis examined trabecular and cortical bone separately, demonstrating most of the effect in the trabecular bone in both types of the treated group. There was increased bone volume of the trabecular bone in both mice types. Before treatment the bone geometry was smaller in the Brtl mice than the wt mice, this did change with treatment but not significantly. There was increased bone deposition in the cortical bone resulting in an ellipsoid shape of the femoral diaphysis in the Brtl mice and a round shape in the wt mice. The mechanical tests, which involved four-point testing of whole bone, found increased bone mass, and ultimate load in both treated groups. However the bisphosphonate treatment did not prevent an increase in the post-yield displacement of the load-deformation curve, which resulted in increased brittleness in both groups, being worse in the Brtl mice. In the treated wt group, the osteoblasts demonstrate an increased response to treatment, however the treated Brtl mice had an altered, potentially toxic morphology of their osteoblasts.

The use of bisphosphonates in children with OI in uncontrolled trials has demonstrated increased bone mineral density, increased vertebral area and increased overall function. However it has not been shown to reduce fractures or bone pain as bone mineral density does not necessarily correlate with bone strength. A randomised controlled study has been recently carried out using olpradronate. The olpradronate study was a 2 year randomised study with 34 children either in placebo or treatment groups. It showed no change in patient ambulatory function or height and vertebral area. It did reveal increased spinal BMD and bone mineral content (BMC). In the present study, eighteen children were randomised to treatment (iv pamidronate every 3 months or placebo) for 18 –33 months. The treated group experienced an increase in the L1-L4 DEXA z-score and vertebral height compared to controls, however there was no effects on long bone length, ambulation and pain. There was a variable response to treated children with regard to vertebral mass and BMD. The DEXA z-scores all
improved in the first year of treatment and then the effects levelled out.

Therefore these studies suggest that the use of bisphosphonates to treat OI in children may have the most effect on the spine rather than the long bones and that the positive benefits are most realised in the first year of treatment. Therefore treatment needs to be limited to 2-3 years and the best time of administration is generally when the patients are from 2-8 years old.

**Saturday 10th July - Plenary Session 2:**
**Chairs: Francois X. Maquart and Peter Brukner**

**David Hulmes** (Lyon, France) started the session by giving an overview of “the mechanisms of matrix assembly-from molecules to tissues”. The ECM is composed of numerous matrix molecules. Each matrix molecule interacts with many others. Consequently the 3-D structure of these molecules is vital to their interactive functions within the tissue. Using the collagen family as an example we can look at the necessary tertiary structures of these proteins.

Specific sequences along the molecule each have a specialised role. Of particular interest is the repeated sequence in the pro-collagen molecule, known as the coiled-coil motifs. These repeated sequences are found in the C-propeptide regions and it is the coiled-coil sequences that can trimerise collagens. There are known to be coiled-coil sequences in most collagens.

So within this 3-D structure and the roles of the coiled-coil sequences within the pro-collagen molecule the removal of C-pro-collagen has a part to play. The removal of the C-pro-component of the molecule causes the mature collagen to begin self-assembly. This leads us to the roles of pro-collagen proteinases. These enzymes function to stabilize the collagen molecules (this also occurs with elastin). These proteinases have a role in the formation of basement membranes, involving laminin 5 and pro-collagen VII.

There are also pro-collagen proteinase enhancers (PCPE’s). They have no enzyme activity, their function is a simple enhancement, by up to 20-fold. By chemical cross-linking, the stoichiometry of the PCPE/C-propeptide interaction was found to be 1:1 in accordance with enzyme kinetic data. In conclusion, although it has been shown that pro-collagen molecules and their various domains can control stages of collagen assembly the case in vivo will undoubtedly be more complex.

**Anders Aspberg** (Lund, Sweden) followed with a description of proteoglycan lectins and their 3D structure with the associated implications for function. The lectin family members include aggrecan, versican, neurocan and brevican. Aggrecan distribution includes connective tissues and the CNS. Versican is almost ubiquitous, whereas neurocan and brevican are both found in the CNS. Each of these proteoglycans has a G1 domain at one end and a G3 at the other. The presence of a C-type lectin globular domain at the G3 terminal characterises these proteoglycans. Also within these domains are one or two epidermal growth factor-like domains and a complement regulatory protein (CRP) domain. The ligands of these proteoglycan lectins include tenascin R and C and fibulin 1 and 2 which all bind to the lectin domain. It is important to note that these interactions are protein-protein interactions not carbohydrate-protein interactions normally expected by C-type lectin domains. These interactions are involved in matrix assembly and have been found to be calcium dependant. For example aggrecan cross-linked by fibulin-2 (dimer) helps to organise other proteoglycans within the ECM. Splicing experiments of the G3 domain in aggrecan have shown 5 different affinities for their ligands, though full length G3 domain remains the most common and best.

The protein-protein interactions occur through fibronectin type III domains on the tenascin molecules. There are three FN III domains 3, 4 and 5. Complexes of aggrecans c-type domain and tenascin R FN III 3-5 were crystallized. Using MIRAS data (heavy atom derivatives), they found that calcium ion coordination in aggrecan lectin domain involves two. Experiments showed that the third FN III domain is not involved in binding, it is only the fourth and fifth.

The lectin-binding surface of aggrecan is very large compared to that of carbohydrate binding surfaces. This means there is a high affinity for interactions. You can divide the surface into three. These are the hydrophobic domain, which has a surface loop and 2 conserved phenylalanines, the CC loop and the fibronectin III domains 4 and 5. The fibronectin III domains have extensive hydrogen bonding and salt bridges. However, there is no direct interaction between tenascin and
the calcium ions. So, what is the role of calcium in the binding of carbohydrate ligands and the C-type lectin domain? It is possible that they function to hold the C-type lectin folded correctly. Experiments crystallising the complexes showed that without calcium the lectin folding was the same. The region that differs is the phenylalanines. The ‘loop’ shape, usually held close to the molecule, becomes flipped out into solution. It is shifted away from the ligand pocket. Another surprise was that calcium was not important in the fibronectin III 4 and 5 binding site.

To conclude ECM ligands of proteoglycan lectins are calcium-binding dependant, however carbohydrate interactions are not coordinated by calcium. So the carbohydrate binding may modulate the protein-protein interactions of lectins and tenascins.

**Martin Humphries** (Manchester, U.K.) presented convincing evidence on the “Cooperation between integrin and syndecan signalling during cell migration”. The formation of focal adhesion sites during cell migration are highly complex, and to date integrins have been identified as key mediators in this pathway. When cells form adhesion contacts on fibronectin several cytoskeletal proteins are involved including actin and vinculin. This process is initiated by the extension of F-actin struts onto the substrate (propagated by cdc 42), which then forms an actin ruffle (Rac 1 signalling) and focal complexes are generated. These focal adhesions change to fibrillar adhesions which initiate the association of actin with the fibronectin substrate. Adhesion mediated by fibronectin is dependent on the interaction of integrin with syndecan 4 located at the focal complex. Additionally the activation of this integrin signalling pathway is dependent on the GTPases cdc 42, Rac 1 and Rho A. Evidence was presented demonstrating that cell migration occurs via activation of Rho A, approximately 1 hour after cell adhesion to a substrate, which was shown to occur after Rac activation.

Various recombinant fibronectin fragments were generated including the 50KDa fragment, fragments which selectively bind syndecans and a C-terminal fragment in which the arginine and lysine residues have been mutated to serines. Data was shown demonstrating that adhesion of cells on the 50KDa fibronectin fragment promoted cell spreading but poor focal contacts were formed. Addition of integrin and soluble syndecan 4 returned the cells to their normal phenotype; without syndecan 4 the cells were seen not to spread. Plating of cells onto the fragment demonstrated normal cell spreading thereby concluding that the fragment is not dependent on the presence of syndecan 4. It was previously reported that there is an 8-fold increase in PKC phosphorylation upon addition of syndecan 4, but this effect was not observed when cells were plated on any of the fibronectin fragments. To investigate this further, melanoma cells were transfected with a dominant negative form of PKC, plated onto the fibronectin fragments and the scratch wound assay performed. Data was presented showing that cell migration was blocked when PKC transfected cells were plated onto the 50KDa fragment; this effect was not observed when plated onto the fragment demonstrating the requirement of integrin, integrin and the recruitment of syndecan 4 and PKC for cell migration. It is believed that integrin signalling is via recruitment of vinculin and not the syndecans. Confocal microscopy was used to demonstrate that addition of syndecan 4 to cells plated on the 50KDa fibronectin fragment produced vinculin focal complexes within 10 minutes of adhesion. Within 30 minutes focal adhesions had formed, and these temporal effects were attributed to Rho GTPases. This was confirmed by adding syndecan 4 to cells plated on whole fibronectin. No effect was observed on cdc 42, whereas there was a transient increase in Rac 1 activation after 10 minutes followed by increased Rho signalling at 60 minutes (initially suppression was noted at 10 minutes). P190 Rho GAP localises to the membrane during initial signalling events (~10 minutes) as less p190 Rho was extractable from the cells. A 2-fold increase in p190 Rho phosphorylation was also observed which was attributed to syndecan involvement at the membrane. As a comparison, normal immortalised fibroblasts adhering to fibronectin were shown to result in a reduction of Rho A as cells start to spread, but after the formation of stress fibres Rho A was observed to increase. Activation of Rac 1 was observed in these cells within 60-90 minutes of plating on whole fibronectin, but this is dependent on the presence of integrin as without it cells remained flattened and there was no Rac 1 activation. Conclusions drawn from this data indicate that
integrins have a differential dependence on syndecan 4, and that syndecan 4 drives the classical Rho family signalling pathway.

After the coffee break, Taina Pihlajaniemi (Oulu, Finland) talked about the roles of type XVIII collagen and its endostatin domain in normal and pathological situations. The two homogenous collagen types XV and XVIII form a subgroup among the non-fibrilar collagens. Type XV is a chondroitin sulfate proteoglycan and type XVIII a heparin sulfate proteoglycan. Elucidation of the complete primary structure of the mouse collagen chain revealed it to be homologous with the previously identified type XV collagen located in the epithelial and vascular basement membranes, and it has been suggested that type XV and XVIII collagens together form a subgroup of multiplexins (multiple triple-helix domains with interruptions) within the collagen family. The function and significance of type XV and XVIII collagens are not known, but a 20-KDa proteolytic fragment called endostatin that is released from the C-terminus of the [1] collagen chain has been shown to inhibit endothelial cell proliferation, angiogenesis and tumor growth. Analysis of patients afflicted by the recessively inherited human autosomal disorder, Knobloch syndrome, involving high myopia, vitreoretinal degeneration, macular abnormalities and occipital encephalocele, revealed mutations affecting type XVIII collagen.

Mice lacking either type XV or type XVIII collagen were viable and fertile but the Col15a1/− mice suffered from skeletal muscle and cardiac defects, whereas in Col18a1/− mice the postnatal regression of hyaloid vessels was delayed, the outgrowth of the retinal vasculature was poor and the vitreal matrix separated from the inner limiting membrane, indicating that this collagen type is important for anchoring the vitreal collagen fibrils to the inner limiting membrane. Further work has demonstrated that lack of type XVIII collagen can result in a variety of phenotypic alterations that are dependent on the genetic background. Additionally, endostatin over-expression was found to markedly affect the lens and skin, leading to lens opacity and a broadened epidermal basement membrane.

Based on the location of type XVIII collagen molecules in the basement membranes and the findings in the Col18a1/− and endostatin over-expression mice, a model was proposed for the role of type XVIII and its endostatin domain in the organization of basement membranes.

Dieter Reinhardt (Luebeck, Germany) provided an overview of fibrillins: structure and function in health and disease incorporating recent data and current hypotheses. Microfibrils are supramolecular aggregates that form extensive fibres in extracellular matrices. Fibrillin is a major structural component of microfibrils present in all tissue extracellular matrices. Fibrillin has 3 family members and these form the backbone of microfibrils. All the fibrillins contain up to 40, Ca^{2+} binding epidermal growth factor (EGF)-like modules. The dominant negative inheritance of a range of diseases called microfibrilopathies is caused by many mutations in either the human fibrillin-1 or the fibrillin-2 gene. Mutations accumulate within the Ca^{2+} EGF-like modules, resulting in either reduced calcium binding or affecting disulphide bonding via altered cysteine residues. Fibrillin-1 mutations in the centre of the molecule can cause neonatal death and Marfan’s Syndrome, which is characterised by long bone overgrowth, defects in the lens of the eye and cardiovascular abnormalities. Immunohistochemical analysis of skin from a Marfan’s patient using a fibrillin-1 antibody showed no structural network, disturbed biogenesis and severe structural instability within microfibrils.

It is thought that perhaps the binding of calcium protects fibrillin-1 against proteolytic digestion, therefore mutations that reduce calcium binding lead to extensive degradation of the fibrillin-1 molecule. Expression of recombinant fragments of fibrillin-1 determined that both wild type and mutated fragments can fold correctly showing no differences in primary structure by rotary shadowing and only slight differences in secondary structure. All fragments were secreted suggesting that during disease abnormal molecules can aggregate and are incorporated into the ECM resulting in unstable microfibril structures. Mutation sites within fibrillin-1 are found close to enzymatic cleavage sites and may increase the susceptibility of fibrillin to digestion, thereby proposing a mechanism that may be important in the pathogenesis Marfan’s syndrome. These cysteine mutations were expressed in fibrillin-1 fragments to determine if enhanced degradation could occur. The fragments were all secreted and showed no structural differences to the wild type fragments by rotary shadowing, again suggesting the abnormal fibrillin-1 is
incorporated into the ECM. For one of the mutated fragments, there was an increase in the number of fragments from the mutated construct and a proteolytic cleavage site was integrated close to the mutation site. This data suggests that proteolytic cleavage may indeed contribute to the pathogenesis of Marfan’s syndrome.

How fibrillin monomers assemble into microfibrils remains unclear. The N-terminus and C-terminus are close to each other and form a parallel head to tail arrangement that may be staggered. Solid phase binding assay and surface plasmon resonance has determined that the N-terminus and C-terminus of fibrillin-1 have a high binding affinity, with only the N-terminus mediating self interaction. Further analyses of homotypic and heterotypic interactions determined that N- to C-terminal binding occurs between fibrillin-1 and fibrillin-2 but no interaction was detected between the termini of fibrillin-2. There are 3 binding sites for heparan sulphate within fibrillin-1 that are involved in self assembly and binding to proteoglycan-associated heparan sulphate is thought to be an important step in fibril formation.

Transgenic mice that lack the proteoglycan, perlecan, exhibit reduced microfibrils, which is consistent with the co-localisation of perlecan and fibrillin to basement membrane structures in the skin and the eye. In addition, perlecan has been shown to interact with fibrillin at a number of different sites. Fibroblasts isolated from the syndecan-4 knock out mouse express an increased fibrillin-1 network in the matrix, however, the cell binding affinity for fibrillin-1 is decreased when compared to wild type cells. It is therefore possible that fibrillin-1 binds to syndecan-4 on the cell surface. The interactions between fibrillin-1, perlecan and syndecan-4 may represent a microfibril assembly mechanism, the structure of which is compromised when abnormal fibrillin molecules are incorporated into microfibrils.

Anthony Weiss (Sydney, Australia) described the molecular and cellular interactions involved in building synthetic elastin. Elastin is an extracellular matrix protein that is very important in tissue biomechanics. It comprises 57% of the dry weight of the aorta, 50% of elastic ligaments such as the ligamentum nuchae, 32% of vascular vessels, 5% of skin and 4% of tendon. However there is little research to date on its involvement in tissue engineered grafts in these tissues. The authors have made synthetic elastin by chemically cross-linking human recombinant tropoelastin in large moulds with a cross-linking agent and amine reactors at 37°C. The resultant material is elastic if wet and its physical performance is like native elastin, being extensible by 200-370% when stretched.

The specifics of assembly involve matured nano-assemblies of tropoelastin, which can gather and fuse into larger elastin fibrils. The overall framework is then filled in with laminin elastin droplets using lysyl oxidase as a cross-linking agent, leading to the solid sheet-like appearance of the material. The synthetic elastin sheets have been modified to be solid on the lower surface and an open weave structure at the top surface so that cells can invade the structure in vitro/in vivo. Synthetic elastin has a similar appearance to native elastin when stained with haematoxylin & eosin, orecin, VVG stain and when examined with scanning electron microscopy. It supports growth of adherent cells in vitro and in vivo; a monolayer of cells will adhere to the smooth surface of synthetic elastin and will encourage growth within the lattice framework. However little is known regarding the targeting of specific lysines in the cross-linking of synthetic elastin compared to native elastin.

Tropoelastin has 35 lysines spread across 17 domains and the lysines are normally arranged in pairs and in hydrophilic domains, which can be either alanine (KA) or proline (KP) enriched. Using mass spectrometry, the individual cross-link interactions with tropoelastin can be identified. In bovine neck elastin, the last third of the allysine aldol cross-link has been found to be lysine enriched and has a great affinity to domain 12/13 of tropoelastin. Several cross-linking domains are not used and lots of interactions are evident at the end C terminus. The lysinorleucine cross-link behaves similarly with domains 4-19 of tropoelastin participating in cross-linking. In synthetic elastin, the lysyl oxidase cross-links are specific for the C terminus. The intramolecular cross-links usually bind between domains 4-15 and there are lots of interactions for intermolecular cross-links at domains 18-25. This region is beside a protease sensitive site. The C terminus-binding site is near to the integrin binding site, this binding site is coded for by exon 36.

Therefore the proposed assembly steps for synthetic elastin are as follows, tropoelastin is
chaperoned to the cell surface by a chaperon protein MAGP-1, the N terminus first binds (domains 6-8, 36) and Integrin (I,II) binds to the C terminus. A 1μm elastin droplet accumulates at the cell surface and is delivered to the tropoelastin.

Sunday 11th July – Plenary Session 3
Chair: Jack Cleujsens

Yoshifumi Ninomiya (Okayama, Japan) opened Sunday’s plenary session with an overview of the three different types of basement membrane: epithelial, muscle and glomerulus and their function in early development, neuronal outgrowth, cell adhesion and migration. His talk focused on the isoforms of type IV collagen which play functional roles in basement membrane assembly and their molecular interactions via the NC1 domain. Using specific monoclonal antibodies he showed the NC1 hexamers and alpha (IV) chain compositions in the glomerular basement membrane which sits between epithelial cells. He showed that the major collagen IV in the choroid plexus basal lamina was the alpha, 3, 4, 5 isoform and hypothesized that the supramolecular network containing the alpha 3, 4, 5 molecules may function as a permeable selective barrier. Interactions were also shown between the alpha 1, 2, 1 and alpha 5, 6, 5 isoforms.

The recently discovered immunoglobulin superfamily of proteins, limitrin, were introduced. Limitrin is expressed at the superficialis and perivascularis where the blood brain barrier functions, but it is not expressed in the median eminence. Disruption of the blood brain barrier by cold injury caused a decrease in limitrin expression, upon recovery limitrin expression returned. Preliminary data showed that the transmembrane domain of limitrin is localized within astrocytes and the extracellular domain is associated in the basement membrane. The lecture also described two types of basal lamina identified in the anteroventral hypothalamus, the anastomotic basal lamina and fractone which is localized to the subependymal layer of the lateral ventricle in the brain.

Nikos Karamanos (Patras, Greece) delivered an in depth lecture on glycosaminoglycans and proteoglycans. He described in detail the methods used to analyse GAGS and proteoglycans and provided some background information regarding glycoconjugates and the glycosylation of proteins. HPLC and capillary electrophoresis were discussed in detail and the benefits of these methods compared. Capillary zone electrophoresis is where solutes migrate in discrete zones at different velocities. ECF is the driving force in capillary zone electrophoresis; consequence of the negatively charged capillary wall double layer. Capillary zone electrophoresis can be used to identify malignant mesothelioma by analysis of pleural effusions. Microemulsion electrokinetic capillary chromatography (MEEKC) was discussed as being a useful method to measure chondroitin sulphate and dermatan sulphate changes in atherosclerosis and the development of human abdominal aneurysm. Measurement of GAG content and sulphation patterns can also be used to diagnose neoplasias of the rectum and pancreatic cancer. The highlighted benefits of capillary electrophoresis over HPLC were demonstrated by the ease and speed of separations and the higher specificity provided by capillary electrophoresis. However, HPLC is still a necessary method for the sequence analysis of GAGs. Final conclusions stated that advances in research requires that we ask the best questions and use the best tools to answer them, and that new tools means new questions.

Adriana Albini (Genova, Italy) highlighted the importance of angiogenesis in tumor formation, invasion and metastasis. She presented the concept of angiogenic switch, in which the overall balance between pro- and anti-angiogenic factors alters the angiogenic response. Results of a number of anti-angiogenic drugs such as bevacizumab (also called Avastin) in clinical trials were presented. The talk then focused on chemopreventive drugs with anti-angiogenic properties such as N-acetyl-cysteine, epigallocatechin-3-gallate (in green tea), 4-hydroxyfenretinide and β-lipoic acid. Some inhibited metalloprotease activity, human umbilical vein endothelial cells (HUVEC) migration/invasion or endothelial cell differentiation in vitro. Some of these drugs affected HUVEC morphogenesis on Matrigel sponge assay in vivo. These components also affected the inflammation response. Screenings of Affymetrix GeneChip arrays showed that the studied chemopreventive drugs affected only a few of the 1500 genes involved in angiogenesis and only 11 genes were common to the angiogenesis and the inflammatory response
including selectin-E, FGF, uPA, COX. Therefore, reduction of both inflammation and endothelial activation could explain the preventive effects observed for the chemopreventive drugs.

**Monday 12th July – Plenary Session 4**
Chair: Bruce Caterson

**Antonio Tamburro** (Potenza, Italy) dissected human tropoelastin, discussing the molecular structure, the self-assembly and the elasticity mechanism. Elastin is a key extracellular matrix protein found within skin, lungs, bladder, elastic cartilage and arteries. It is principally synthesized during the development or growth of tissues, with tropoelastin expression occurring during mid-to late fetal or embryonic periods. Besides being responsible for the elastic properties of connective tissue, it is known that tropoelastin and some soluble derivatives of elastin exhibit biological activities including the ability to modify cellular behavior. Several elastin-derived peptides have been identified which show chemotactic activity for monocytes, fibroblasts and for certain tumoural cells. The importance of the major properties of elastin to the field of tissue engineering has been illustrated by recent biomaterial designs, which have focused on the use of artificial biodegradable elastomers and acellular elastin matrices.

The elastin gene is a single copy gene localized in chromosome 7 in humans and, under normal conditions, is expressed by various cell types during the pre- and neonatal stages of development. The elastin gene product, tropoelastin, is a protein of 750 to 800 residues, and owing to numerous cDNA analyses, its full sequence is now known for the human, bovine, chick, rat, mouse and sheep species. Tropoelastin is synthesized and secreted by smooth muscle cells and fibroblasts. Secretion is followed by an orchestrated interplay of macromolecular partners that assist in delivering tropoelastin monomers to sites of elastogenesis. Such interactions facilitate identification of sites for elastin assembly through associating microfibrillar proteins and encourage deposition with previously accreted tropoelastin. Conversion to elastin is made possible by the action of lysyl oxidase, which converts the epsilon amine on side chains of occasional lysines in tropoelastin to the adipic semi-aldehyde. Coacervation, juxtaposes modified and unmodified lysines to facilitate irreversible covalent cross-linking. In human elastin, at variance with other species, there is a rarely expressed exon (26A), which is involved in alternative splicing. In contrast to the rest of the protein, the sequence coded by this exon is rich in charged and polar amino acids.

Only limited information is presently available on the properties of elastin containing this additional sequence and on the biological activity of the peptides derived from this exon. Conformational studies demonstrated the presence of labile conformations, whose stability was found to be strongly dependent on the microenvironment and provided an experimental basis to the understanding of the molecular mechanism of the elasticity of elastin. Further work, gave significant insight to the roles played by specific polypeptide sequences in self-assembly and possibly elasticity.

**Alain Mauviel** (Paris, France) reviewed the current knowledge regarding the transcriptional control of fibrillar collagens, namely collagen type I, by TGFß and the inflammatory cytokine, TNFα. The importance in the balance of these two factors in development, homeostasis and tissue repair were discussed. The SMAD signalling pathway downstream of TGFβ activation was described and the resulting up regulation of type I collagen gene transcription. TNFα activation causing down regulation of the collagen I gene through either activation of the NF-κB or MAPK/JNK pathways was also explained.

Interest in the regulation of TGFβ is a result of its implication in the development of fibrotic disorders. In depth knowledge of these interactions are of potential therapeutic interest in the context of fibrosis. Pro-inflammatory cytokines do inhibit the TGFβ / SMAD pathway regulating Col1A1 gene expression. This has been shown to be JNK dependent and through direct inhibition of SMAD / DNA interaction. Confirmation of the role of JNK was through use of JNK knock-out fibroblasts and expression of JNK1 in a mammalian two hybrid system. Absence of SMAD / AP-1 heterocomplexes on DNA was confirmed by gel shift assays. The lecture was concluded by summarising the importance of JNK in inhibiting the SMAD / DNA interaction and for c-jun to antagonise TGFβ signalling.

**Sarah Rees** (Swansea, UK) demonstrated the importance of aggrecan catabolism in tendon.
Tendon is composed predominantly of type I collagen, however, proteoglycans also modulate the structural and material properties of the tissue. Variations in the proteoglycan and/or GAG content of tendon have been observed both with age and during disease (tenonitis) but the mechanism of degradation (catabolism) of proteoglycans in tendon was previously unknown. This presentation by Dr Sarah Rees described the differential expression profiles of aggrecan and aggrecanase in the bovine deep digital flexor tendon obtained from experiments using western blotting and RT-PCR.

Experiments were conducted using tendon samples from both young and mature animals and also, from different regions of the tendon that are normally subjected to compressive and tensile forces. Tendon proteoglycans include aggrecan, decorin and biglycan. Although the functions of tendon proteoglycans are not fully understood, changes in their turnover are associated with tendinopathies. Aggrecan degradation in tendon is associated with cleavage within the intraglobular domain at the aggrecanase site (Glu373 – Ala374). Aggrecanases (ADAM-TS4 and -5) were found to be constitutively expressed and active in tendon, indicative of a high rate of aggrecan turnover. This is in marked contrast to the expression and activity profiles of the aggrecanases in articular cartilage.

The data presented indicates that aggrecan turnover in tendon is approximately 3.3 fold higher than in articular cartilage. In addition, data showed that components of the aggrecan proteoglycan aggregate are extensively catabolised in tendon under constitutive conditions and that aggrecan is present in between tendon collagen fibres and fibril bundles. This study has provided insight into the role of aggrecan and its distinct physiological properties in tendon. As the collagen fibrils undergo extension, aggrecan may dissipate resultant compressive loads by resisting the flow of water in these locations. In addition, aggrecan may facilitate the sliding of fibrils during the small amount of elongation of the tendon whilst under tension. Thus, the half life of aggrecan may be significantly reduced because it constantly participates in repeated resistance to compression.

Importantly, inhibition of the tendon aggrecanase activities, which are required to maintain normal metabolic homeostasis, may explain the occurrence of musculo-skeletal side effects in clinical trials investigating the use of matrix metalloproteases inhibitors to slow or halt articular cartilage destruction. This study highlights the difficulties in designing therapies based on MMP inhibition as there is differential expression in connective tissue, which may reflect their function.

Tuesday 13th July – Plenary Session 5
Chair: Guido David

Complementing M. Humphries talk, and starting the session was Pascale Zimmerman (Leuven, Belgium) who presented her work on “Cytosolic and membrane components involved in syndecan trafficking and signalling”. The well characterised syndecans are sugar chains linked to protein cores which are involved in extracellular ligand binding to conserved intracellular domains contained within signalling and cytoskeletal proteins. They function in modulating cell adhesion and growth factor signalling. Interaction studies were previously used to confirm the association of the C2 domain of syndecan 2 with the PDZ domain of syntenin; the PDZ domains are involved in cell sorting, targeting and assembly of supramolecular complexes. It was also previously demonstrated that the PDZ domains of syntenin interact with PIP2 at the membrane surface.

To understand the functional relevance of syntenin binding to PIP2, constructs of syntenin mutants were generated whereby either the C- or N-terminal end of the PDZ domain was deleted. Data was shown demonstrating that the mutated forms of syntenin could not bind PIP2 but were still able to bind syndecan 2. In the cells containing the mutated syntenin, syndecan 2 was shown to localise as concentrated pools in perinuclear structures; this was in comparison to confocal images showing syndecan 2’s normal localisation in pericellular regions and at the cell membrane. Interestingly this abnormal pattern of syndecan distribution was observed in all of the mutant syntenins, thereby independent of whether the C- or N-terminal regions were absent. Data was presented which demonstrated that a chimera of the mutant constructs rescued the phenotype suggesting that the phenotype is dependent on the PDZ domains. Confocal images were shown to indicate that syntenin and syndecan 2 do not co-localise with Golgi or endoplasmic reticulum markers but with lysosomal markers suggesting an accumulation of syntenin-syndecan 2
complexes in the lysosomes. Dr Zimmerman presented data demonstrating that syndecan 2 is endocytosed by using an anti-syndecan 2 bivalent antibody and tracking the uptake of the antibody. After 1 hour there was partial uptake by the lysosomes which was complete by 4 hours.

To investigate the mechanism of endocytosis further, the non-classical clathrin pathway (Arf6) was analysed. Arf6 is a GTPase which alters actin dynamics, and as such activation can lead to protrusions, spreading, migration and differentiation. Arf6 is subject to cycles of activation which triggers movement/trafficking of molecules to the plasma membrane. As the mutated syntenin-syndecan 2 complexes accumulate in lysosomes, Arf6 constructs were generated to determine what the underlying mechanism is. A dominant positive Arf6 mutant resulted in blocking of transport in the early endosomes, whereas a dominant negative mutant resulted in inhibition of sorting and recycling to the endoplasmic reticulum. Thus, in the dominant negative form it was demonstrated by confocal microscopy that the syntenin complex accumulated in perinuclear regions, therefore it was suggested that syntenin controls the exit of PIP2 by Arf6 recycling.

Final experimental evidence indicated that the syntenin-syndecan complex is essential for cell spreading, as there was a 2-fold decrease when the mutants were placed on heparan sulphate. This characteristic loss of cell spreading was confirmed using siRNA to abolish syntenin expression. The final few minutes were used to present ongoing studies including abrogation of syntenin in zebrafish which appeared to generate a lethal phenotype. Also syntenin +/- embryos generated using morpholino oligos were found to be absent of FGF, BMPs and Wnts expressions, hence demonstrating the importance of the syntenin pathway in cell migration and differentiation.

Following on from this was the SISC “Castellani Lecture” presented by Daniela Quaglino (Modena, Italy) who gave an enlightening talk on the rare connective tissue disorder “Pseudoxanthoma elasticum: how a metabolic defect affects the extracellular matrix”. Pseudoxanthoma elasticum (PXE) is a disease characterised by dermatological alterations including skin relaxation and dermal papules (presenting on arms, neck and legs). Other clinical symptoms include retinal neo-vascularisation (in the Bruchs membranes) and both retinal and gastrointestinal haemorrhages. PXE is an inherited disorder, and the gene responsible is located on chromosome 16 encoding a protein called MRP6. MRP6 belongs to the ATP-binding cassette membrane transporters, although its physiological role and substrate are unknown. Many mutations have been described for this gene which are all located in exons 24 – 30.

As the disease name suggests, the neo-vascularisation is attributed to elastic tissue mineralisation; PXE is initially diagnosed by Von kossa staining of the mineralised tissue. Mineralisation of the elastic tissues are widespread but predominantly occur in the heart and lungs resulting in abnormal matrix deposition including lateral fusion of the collagen fibrils and aggregation of dense material rich in glycosaminoglycans with differing sulphation properties. Radiolabelling and ion-exchange chromatography was used to demonstrate differences in PXE fibroblast PG synthesis; chondroitin sulphate was shown to decrease whilst there was an increase in both production and secretion of heparan sulphate. Increased proteolytic activity was also observed in the PXE fibroblasts with enhanced activation of the elastases and pro-MMP 2 in cell culture.

Using SEM, PXE dermal fibroblasts were shown to have enlarged endoplasmic reticulums and were observed to contain many nuclei within an individual cell. Using in vitro assays they further investigated the enlarged cells by analysing cell volume using flow cytometry. PXE fibroblasts were approximately 1191 in comparison to a volume of 744 in normal dermal fibroblasts; PXE cells were also demonstrated to have a higher proliferative capacity. To understand the PXE cell phenotype, collagen gel retraction experiments were performed and data was presented indicating the inability of PXE fibroblasts to contract the gels when compared with normal fibroblasts. Adhesion of these cells on different substrates including plastic, type I collagen and fibronectin showed a much slower attachment. Interestingly fibroblasts removed from unaffected sites of a PXE patient also demonstrated reduced attachment compared to normal fibroblasts. The reduced adhesion properties were analysed by examining integrin expression. A combination of confocal imaging and flow cytometry demonstrated that there was decreased expression down-regulating both and integrin complexes, although 😊.
integrin expression was increased. Although changes in the cytoskeleton were analysed, there was no effect on either actin or vimentin localisation. The conclusion derived from this talk was that PXE is a metabolic disorder whereby a mutation in the MRP6 protein prevents correct trafficking of molecules thereby creating an abnormal extracellular matrix in the soft connective tissues. Proteomic analyses are currently being undertaken to determine the complex network that gives rise to PXE.

Henning Langberg (Copenhagen, Denmark) discussed his many years of work and the pioneering technique using in vivo microdialysis to evaluate changes in the peritendinous regions of the human Achilles tendon in response to exercise. Overuse tendon injuries account for up to 50% of all sports injuries, the cause and treatment of most remains unknown. Previously published data has shown that local markers of collagen synthesis increase with both acute and prolonged training. This study assessed the effects of 1 and 3 hours running on type I collagen formation and growth factors in peritendinous tissue in adult human males. Other previous studies have looked at tenocytes’ response to load compression and strain in vitro. In vitro studies may be oversimplistic considering the many factors that have been identified that contribute to tendon turnover, including hormones, cytokines, signals from surrounding cells (synovial sheath vascularisation), adipose tissue and load.

The main findings presented showed that in the achilles tendon, peritendinous blood flow is increased during both static and dynamic exercise. This is accompanied by increases in certain growth factors and cytokines and an increase in the formation of type I collagen. Therefore, with regards to circulation, metabolism and collagen formation, peritendinous tissue represents a dynamic, responsive region that adapts markedly to acute muscular activity.

Plasma concentrations of TGFβ1 rose 30% in response to exercise, suggesting a role for TGFβ1 in mechanical regulation of local collagen type I synthesis in tendon-related connective tissue in vivo.

Tendon, like muscle may adapt to exercise by increasing collagen, therefore increasing size of the tendon. In support, Langberg showed that cross sectional area of Achilles tendon increases in trained subjects and that the Achilles tendon had regional differences in the pattern of peak stress. The exercise-induced responses identified in these studies occur much faster than previously believed, showing an increase of type I collagen synthesis within 24 hours. In addition, newly published data indicate that exercise changes the interstitial levels of MMPs, which may indicate that MMPs have a role in the exercise induced adaptation of the connective tissue.

These are important considerations for those research groups involved in understanding tissue response to exercise and should be taken into account when planning training regimes for racehorses and human athletes.

**POSTER PRIZE LIST**

Poster prize winners were awarded 200 euros each, from the Italian and the French Connective Tissue Societies.

- PM23 - Maria Assouti, Patras, Greece
  **The diagnostic significance of Collagen type IX and HNK-1 epitope in XFS**
  Assouti, M.S., Georgakopoulos, C.D., Gartaganis, S.P., Vynios, D.H.

- PC6 - Valérie Cenizo, Lyon, France
  **Lysyl oxidase-like is associated with elastic fibers and downregulated during skin ageing**
  Cenizo, V., Bouez, C., Gleyzal, C., André, V., Reymermier, C., Thomassin, L., Perrier, E., Sommer, P., Damour, O.

- PK8 - Silke Maier, Cologne, Germany
  **Characterization of SMOCs, modular extracellular calcium-binding proteins**
  Heep, D., Maier, S., Smyth, N., Miosge, N., Hülsmann, H., Frie, C., Paulsson, M., Hartmann, U.

- PA26 - Vasiliki Petropoulou, Manchester, U.K.
  **Structure-function studies of BMP1 (Bone Morphogenetic Protein 1) and TLL2 (Tolloid-like 2): identification of procollagens binding domains**
  Petropoulou, V., Garrigue-Antar, L., Kadler, K.

- PB22 - Fiona Schneiders, Cologne, Germany
  **Biochemical characterisation of Ntrin-4**
  Schneiders, F., Smyth, N., Koch, M.

- OM2 - Annemarie van der Slot, Leiden, Netherlands
Increased pyridinoline cross-link levels in fibrosis is accomplished by TGFβ induced lysyl hydroxylases 2b expression
Van der Slot, A.J., Zuurmond, A.M., Bank, R.A.

BSMB Autumn 2004 Meeting Report, Bristol, Joint With The UK Tissue & Cell Engineering Society

“Cell Based Therapies”
Report by Laure Sudre and Frank Cheung

The autumn 2004 meeting of the BSMB was held in the Wills Memorial Building at The University of Bristol on the 13th-14th September. The meeting was held jointly with the UK Tissue & Cell Engineering Society (TCES) and its theme was “Cell Based Therapies”. The meeting was organised by Professor Anthony Hollander and Dr. John Tarlton, and was supported financially by Europa Bioproducts and Sigma and was sponsored by Kendro, Fisher Scientific, Thermo, SciQuip, Genzyme, Invitrogen, Leica, Geistlich, R&D Sytems, Cellon S.A., Verigen, Promega, SLS, Sigma-Aldrich, Blackwell Medicine and Amaxa Biosystems. There were 13 invited speakers: two from the USA, five from continental Europe (Sweden, Italy and Switzerland) and six from the UK. A further nine short presentations were selected from the submitted poster abstracts. The meeting attracted 150 registered delegates.

Dr Robert Nerem opened the meeting with his talk on Vascular Tissue Engineering: Cells, Matrix, and Immune Acceptance. He addressed the necessity to create small diameter blood vessel tissue. To achieve this, there are critical issues that need to be addressed. The cell source is critical as differences between different endothelial cells can be observed (like the expression of polarity, extracellular matrix and signalling pathway genes). In other words, “an endothelial cell is not an endothelial cell, is not an endothelial cell”. The matrix is also critical as it provides important cues for the cells and different matrix can result in differences in signalling. It is also important to know how much a tissue engineered biological substitute has to be “matured” in vitro prior to implantation. If off-the-shelf availability is to be achieved, then a suitable cell source for an endothelial lining must be established and immune acceptance must be engineered, which may represent the biggest challenge.

Prof Anders Lindahl discussed Autologous Cartilage Implantation (ACI). Articular cartilage is unable to repair itself, due to the lack of innervation, blood supply and because of the encapsulating matrix surrounding the chondrocytes. Therefore, could it be transplanted with cultured chondrocytes? ACI has been used since 1987 in humans and since then, over 12000 persons have been treated. Clinical results are promising. Furthermore, cultured articular chondrocytes demonstrate plasticity with an ability to form chondrogenic, adipogenic and osteogenic cells. However, development of future therapies in cartilage repair needs to be achieved. Future works will focus on the investigation of the use of embryonic stem cells as a universal donor and the ability to manipulate of cellular pathways.

Dr Brian Ashton gave an overview of different approaches to cartilage repair. In the US, 63% of the patients, who had a non-related knee injury, show sign of cartilage lesion. 41% of patients with cartilage lesions had deep fissuring of the cartilage and 19.2% had cartilage loss. So is it necessary to recreate the articular cartilage to achieve long lasting repair? To date, the standard techniques are articular debridement, marrow stimulation techniques (which accelerate the formation of repaired tissue from mesenchymal stem cells), mosaicplasticity (which consists in removing osteochondral cylinders and forming them into osteochondral defect; but the integration of the graft is not good) and autologous chondrocytes implantation, ACI (which consists of “growing” a patient's cartilage cells and injecting them back into the damaged cartilage). ACI gives good results, but nobody knows how it works. Therefore, a new program is about to start called ACTIVE which will look at ACI versus alternative treatments for cartilage injuries.

Alessandra Pavesio described 3-year clinical results of articular cartilage engineering with Hyalograft® C. Articular cartilage regeneration represents a major challenge. Tissue engineering is a potential therapeutic option and has led to the development of Hyalograft® C. This engineered scaffold is composed of autologous chondrocytes grown on a HYAFF® scaffold. Hyaluronan (HA) is the most conserved molecule after DNA and because it is a naturally
occurring polysaccharide in the body, it is not dangerous. HA scaffolds have both biological and structural roles. HA is important for hydration and compression of cartilage and also has a role in chondroprotection and chondrogenesis. The Hyalograft® C allows the cells to re-differentiate and to lay down an extracellular matrix. Under these conditions, collagen I is down-regulated, whereas collagen II is up-regulated. HYAFF® is degraded without provoking an inflammatory response; therefore it is safe, biodegradable and biocompatible. A study on 192 patients treated with autologous chondrocytes grown on a HYAFF®, with an average follow-up time of 38 months, has shown that 91.5% of patients improved. Biopsy on 21 patients showed cellular re-organisation, lateral integration of the graft and histological analysis showed the deposition of hyaline-like material. In conclusion, Hyalograft® C appears to be safe and can provide an effective therapeutic option for the treatment of articular cartilage lesions.

Prof Paul Halton discussed a range of biomaterial scaffolds for cartilage tissue engineering. Early scaffolds for tissue engineering were based on existing medical polymers, but new evidence suggests that alternative polymers may improve the biological quality of engineered tissue. The SCAFCART project has been developed to look at novel bioresorbable scaffolds and culture methods for cartilage tissue engineering. Analysis of different scaffolds (block copolymer foam and esterified hyaluronic sponge) that have been seeded with chondrocytes, show matrix formation around the periphery and outer regions of the scaffolds with few cells and little matrix in the centre. A non-woven fleece of esterified hyaluronic acid produced good cell penetration and matrix (with hyaline characteristics) distribution throughout the scaffold. Calcium phosphate ceramics and bioglass porous hydroxyapatite encourage new bone formation, tissue formation and direct apposition following implantation. In conclusion, cell seeding and culture conditions need to be optimised for specific scaffolds. Improved performance will be achieved using different approaches: complex or composite scaffold, surface (chemistry, topography, patterning, etc) and biological modifications.

Prof Kevin Shakesheff described the use of novel injectable scaffolds to create an optimised environment around therapeutic cells. Scaffolds need to subdivide large 3D spaces, define surface chemistry, release pharmacological agents, transmit or negate mechanical forces and assist spatial organisation of cells. Stem cells need to be injected in a specific location; a useful approach would be to develop an injectable scaffold containing cells. The self-assembling macroporous scaffold under development contains biodegradable polymer microparticles, cells and a crosslinker or ‘setting agent’ (temperature trigger which promotes gelation). The components can be mixed in a syringe and once injected at the correct location, the surgeon has about 5 minutes to manipulate and mould the tissue before it becomes rigid. Preliminary tests are promising and show that cells adhere to the engineered material, proliferate and lay down a collagen matrix over an 18 day period.

Dr Jean-François Clémence considered naturally occurring biomaterials. A key factor for successful tissue engineering is an appropriate matrix. Bio-Oss® hydroxyapatite crystal structure is similar to human bone and is therefore an ideal template for new bone formation. The surface of Bio-Oss® allows all type of proteins or cells to attach. The matrix structure of Bio-Oss® resorbs only slowly, thereby enabling the augmented bone to achieve stability, and preventing resorption of the newly formed bone. Histological studies have shown that Bio-Oss® increases the mineral content of the regenerated graft. The advantages for the use of Bio-Oss® are: safe and predictable clinical outcome; avoid or decreases dependence on the use of autologous bone grafts and the preservation of bone volume. Bio-Oss® linked to BMP2 has been compared to the use of Bio-Oss® alone. Histomorphometry at 6 months showed there was very little extra newly formed bone with BMP2. Dr Clémence concluded his talk by saying that: the current approach of tissue engineering is rather trivial compared to the complexity of the regenerating tissue; that tissue engineering is showing clinical benefits: and that the value of proper carriers in tissue engineering is underestimated.

Prof Andrew Newby gave an overview of cardiovascular gene therapy. There are different options for achieving gene therapy in vascular tissues: delivery route, vectors, mechanisms and gene targets all have to be considered. To inhibit smooth muscle cells (SMCs) proliferation, an E2F decoy has been used. In phase 1, patients were assigned with E2F decoy-treated artery bypass grafts. The effect of E2F decoy was to inhibit PCNA mRNA and proliferation within the grafts. In the phase 2, results show a reduction in wall thickness and in graft failure. The over-
expression of TIMP3 in human saphenous vein has been achieved using viral vectors. After 14 days, there is an abolition of intima formation, a problem affecting many grafts. TIMP3 binds to the extracellular matrix and hence, even when the host viral vector is no longer present, increased levels of recombinant TIMP3 can still be detected. Adenovirus-TIMP3 (Ad-TIMP3) loaded onto phosphocholine (PC)-coated stents have been delivered in vivo to pig coronary artery, also resulting in the reduction of intima. Ad-IL10 decreased artherosclerosis and vein graft thickening in ApoE deficient mice. In conclusion, E2F decoy is simple and the closest to clinical use. TIMP3 is effective for vein graft treatment and IL10 is a good candidate for helping treatment of artherosclerosis.

Prof. Chris Evans discussed the use of gene therapy in tissue engineering. The impact of modified gene expression via molecular vehicles suggests that long-term expression is probably not required, but may be contingent on the developmental stage of the tissue, environmental factors, etc. The potential benefits of such an approach is expedited tissue repair, requiring no cell culture, no scaffolds, and only single injectable Adenovirus vectors. A brief description of the adenoviral delivery system was discussed, which lacks both the functional E1 and E3 regions. BMP-2 transgene was introduced into a 5mm defect in Sprague Dawley rats, 24 hrs after the surgery which generated the tissue defect. This produced increased bone repair as compared to an empty vector control, with mechanical loading being a suggested mechanism to fully complete the complete remodeling of the repair tissue. Genetic modification has also been used with BMSCs, whereby TGF-beta introduction generated improved chondrogenesis in an animal model (increase Type II, decreased Type I). There was very good localized expression of the gene in the area of the defect, with very little diffusion into the synovium.

Dr. Ivan Martin is investigating the regulation of chondrocyte differentiation and matrix deposition for cartilage tissue engineering. Adult human chondrocytes have a reduced capacity to re-differentiate and generate cartilage tissues. Regulation of human chondrocyte phenotype and matrix deposition may be achieved using growth factors, 3D scaffolds and physical forces. Addition of growth factors in monolayer before 3D pellet culture can guide or programme the chondrocytes. In the presence of growth factors, human chondrocytes can achieve 10 doublings in 12 days instead of the 42 days required by the control. In the presence of 10% FCS + TFP (TGF beta 1, FGF2 and PDGFbeta), the chondrocytes produce more GAG and DNA than in control cultures. The capacity of the cells obtained from donors of different age to differentiate is similar. However, in the presence of TFP, cells from young adults have a higher capacity to differentiate. Variability in the clone capacity to differentiate is observed between different individuals but also between clones from the same individual. Therefore there is a necessity to improve the sorting of specific cell subpopulations. Polyactive (block co-polymer composed of PEGT and PBT, where the concentration in both components can vary) has been used as a scaffold for tissue engineering. Its composition regulates chondrogenesis by expanded human chondrocytes and its architecture modulates chondrogenesis most efficiently if the composition of the polyactive is 70% PEGT, 30% PBT. Future challenges include: improving the properties of engineered cartilage and identifying optimal condition of culture, in order to simplify the production of a uniform quality of engineered cartilage.

Prof. Ranieri Cancedda discussed the use of bone marrow stromal cells, seeded onto biodegradable scaffolds, to repair large bone damage. When implanted into immunodeficient mice, BMSCs seeded onto mineralized tridimensional scaffolds form a highly vascularized primary bone tissue. Bioceramic HA scaffolds with different architecture were used as implants into the defect area of damaged bone. Synchrotron Radiation microtomography (SR Micro-CT), using 3D analysis to measure contrast differences between unseeded and seeded scaffolds, was used to determine scaffold and cell durability and performance after 8-12 weeks. Newly formed bone, as a measure of volume and thickness, was estimated on the two different scaffold architectures. The scaffold with higher bone surface, porosity, higher isotropy, and thinner walls. When implanted into patients, autologous BMSC/bioceramic composites showed full functional recovery within 6-7 months post-surgery. More work is still required in optimizing the source of cells, as autologous cells prevents commercial, off-the-shelf use of the procedure, and may require more study regarding the expression of immunorelevant molecules and cytokines thought to initiate the host’s response to transplants.
Dr Richard Smith described the transplantation of engineered pancreatic islets to treat Type I diabetes, and the use of gene therapy to increase islet survival and insulin release. An obstacle to successful transplantation is the loss of up to 50% of the islets as a result of non-antigen specific inflammatory cytokine release and recurrent autoimmune disease, which cannot be treated systemically because of the resulting toxicity. Another problem is the availability of a replenishable supply of beta cells for islet engineering. Gene therapy in this area has focused on a number of key factors, including VEGF165, AMP kinase and CTLA4-Ig, introduced into isolated islets with adenovirus vehicles. In vitro islet function was assessed by quantifying insulin release in response to 25mM glucose. In vivo functionality was assessed by transplantation into streptozotocin-generated diabetic mice. AMP kinase inhibition proved quite successful, with reduced islet cell apoptosis, and perceptible increased insulin release. In general, the results indicated that gene therapy produced increased islet survival, illustrated by increased total insulin release, but requires more careful study to confirm which molecular targets are the most important prior to full clinical trials in Bristol’s new Southmead Hospital clean labs.

Prof Sheila MacNeil was the last invited speaker and she considered the current state of skin graft engineering, using thin sheets of integrated epidermal or oral keratinocytes and fibroblasts. The commercial use of the engineered tissue has proved quite difficult for a number of reasons, including economic costs and fragility/required sterility of the skin graft, and has prevented the widespread use of such sheets. Attempts have been made to make the use of skin grafts more clinician/patient friendly. This includes the use of chemically-defined plasma coated surfaces onto which keratinocytes can be cultured and delivered to patients with reduced handling and storage, a technology termed Myskin. This has successfully been used to treat patients with non-healing diabetic foot ulcers, as well as severe burns. Xenobiotic-free cell cultures on electrospun 3-D scaffolds is currently underway.
Spring 2005 Meeting of the British Society for Matrix Biology

“Collagen – from genes to fibrils”

To be held on March 21st and 22nd, 2005
at the Sherrington Buildings, University of Liverpool.

The Spring 2005 meeting of the British Society for Matrix Biology will be held in the Sherrington Buildings, University of Liverpool on March 21st and 22nd, 2005. The meeting is organised by Anne Vaughan-Thomas (a.vaughan-thomas@liv.ac.uk; 0151 794 4784), Pete Clegg (p.d.clegg@liv.ac.uk; 0151 794 46077) and Stuart Carter (scarter@liv.ac.uk; 0151 794 44206), who may be contacted for further details. The meeting will include invited speakers from the USA, Europe and the UK, details are given in the programme.

The theme of the meeting is ‘Collagens – from genes to fibrils’ and we have put together a programme of talks given by speakers who are at the forefront of collagen research. However, we have also included a theme-free session, which will give a few junior researchers with an interest in any aspect of matrix biology an opportunity to present their work.

The venue is the Sherrington Buildings on Ashton Street, which were opened in 2002 for teaching Medical Students at the University of Liverpool. Lectures, posters, exhibition stands, catering and refreshments will all be located within the Buildings. The Sherrington Buildings are around 15 minutes walk from the accommodation and Liverpool Lime Street station, which is near the Walker Art Gallery, Liverpool Museum and the impressive St George’s Hall. The registration desk will be sited in the foyer of the Sherrington Buildings and will be open from 11.45 pm.

Accommodation. We apologise that university accommodation is not available within 2 miles radius. All accommodation will be in the extensively refurbished three star Gladstone Hotel (soon to be The Liner; http://www.liner.co.uk.), located adjacent to Liverpool Lime Street Station. Single en-suite rooms are available at £45.00 pppn and twin en-suite rooms are available at £35.00 pppn (Please list accommodation requirements on the registration form and provide the name of the person you are sharing with in order to avoid double booking).

The conference dinner will be in the Woodside Ferry Terminal, which will be reached by taking the ferry across the Mersey. This grade II listed building has unrivalled views of Liverpool’s skyline, the waterfront and Pier Head which received World Heritage Site status from UNESCO in 2004.

Directions. Printer-friendly directions to the Gladstone Hotel can be found on http://www.theliner.co.uk. The Sherrington Buildings are on the end of Ashton Street off Pembroke Place, located next to 25 on the map available at http://www.liv.ac.uk/maps/precinctplan.pdf and a map showing Pembroke Place in relation to Liverpool Lime Street Station is available at http://www.liv.ac.uk/maps/images/city_map.gif. If you wish to drive, free car parking will be available at the Gladstone Hotel for guests. No university parking is available but there is a NCP car park on Mount Pleasant. For information on disabled persons access, please do not hesitate to contact the organisers.

All early-bird registrations will be entered into a free draw for £10 prize. The closing date for early-bird registration is Friday, 28th January 2005. The deadline for the standard rate of registration and abstract submission will be Friday 11th February 2005, after which a late registration surcharge of £5 will be levied. Conference presentation and reporter bursary applications (see details) closing date January 15th. The conference dinner will be available for booking on a first come first serve basis.

Poster and oral presentations may cover any area of, or related to, matrix biology; abstracts do NOT have to be within the meeting theme. All abstracts submitted will be published in the International Journal of Experimental Pathology and there will be a IJEP-sponsored poster competition (for eligible applicants, i.e. PhD students and young post-doctoral workers in their first 3 years) with 3 substantial cash prizes of £150 to be awarded. Poster prizes will be awarded on the basis of votes cast by delegates.

Some abstracts will be selected for oral presentation either within the theme-free session (10 minutes duration + 5 minutes for discussion) or at various points throughout the programme. Speakers will be notified in advance of the meeting.

We look forward to seeing you in Liverpool!
Collagen- from genes to fibrils.
The Sherrington Buildings, University of Liverpool, March 21st/22nd 2005

Provisional programme- Please check website for amendments prior to the meeting

Monday 21st March 2005
Registration in Foyer of Sherrington Buildings- open from 11.30 a.m.

1.10 Welcome

Probing the structure of collagen and organisation of collagen

1.15 Prof. David Hulmes, Lyon
Provisional title: Coiled coils, trimerisation of collagen alpha chains

1.50 Prof. Karl Kadler, Manchester
Cellular aspects of collagen fibrillogenesis

2.25 Prof. Allen Bailey, Bristol
The functional fibre

3.00- 3.30 Tea

Interactions and organization

3.30 Prof. Cay Kielty, Manchester
Provisional title: Collagen VI or VIII organization

4.05 Prof. Vic Duance, Cardiff
Provisional title: Mixed collagen fibril systems

4.40 1 short presentation

5.00- 5.30 BSMB AGM
5.30- 6.30 Wine Reception and Poster session
7.30 Conference dinner

Tuesday, March 22nd

Diversity within the collagen superfamily

9.10 Dr Ray Boot-Handford, Manchester
Provisional title: Evolution of vertebrate collagens- type XXVII collagen

9.45 Prof. Taina Pihlajaniemi, Oulu, Finland
Provisional title: The transmembrane collagens

10.20 1 short presentation

10.40- 11.10 Coffee
11.10- 1.00 Theme-free session

6 Short presentations (15 min each)

1.00- 2.00 Lunch and Posters

**Collagen mutations and consequences**

2.00  Prof. Bjorn Olsen, Harvard, Boston, USA
      Provisional title: Collagen XVIII - transgenes and human mutations?

2.40  Prof. Alan Wright, MRC Human Genetics Institute, Edinburgh
      Provisional title: CTRP5 mutations associated with L-ORD

3.10  Prof. David Birk, Jefferson Medical College, Philadelphia
      Type V collagen regulates fibril assembly: Transgenic mice as a model of classic EDS

3.45  1 short presentation

4.05  Closing remarks, poster prizes and end of meeting
British Society for Matrix Biology
BSMB Spring 2005 meeting, March 21st and 22nd 2005
The Sherrington Buildings, University of Liverpool

COLLAGEN - FROM GENES TO FIBRILS

REGISTRATION FORM
Early-Bird Registrations will be entered into a free £10 prize draw. Closing date: 28th January 2005.
Standard rate Registration closing date: 11th February 2005.
After 11th February, delegates will be charge a late registration surcharge of £5.

Title:…………. Family/Last name:………………………………First name:……………………………...
Correspondence Address (please include postcode):……………………………………………………..
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Tel:……………………….. Fax:………………………… Email:……………………………………………..
Special Dietary Requirements (please specify e.g. vegetarian, none etc):………………………………

REGISTRATION FEES (Fee includes coffee/tea both days; lunch on Tuesday):
Registration for BSMB members £40:-…………..
Registration for Student BSMB members £30:-…………..
Registration for Non-members £50:-…………..
Registration for Student Non-members £40:-…………..
Late Registration surcharge (after 11th February 2005) £5:-…………..

ACCOMMODATION at The Gladstone Hotel (www.theliner.co.uk) (includes breakfast):
20th March ………… 21st March ………… 22nd March: …………
Single occupancy with en-suite facilities, £45 per night: …………
Twin room with en-suite facilities, £35 per night: …………
Please provide the name of the person you are sharing with: …………………………………

CONFERENCE DINNER:
Monday 21st March, Woodside Bistro (including ferry across Mersey) : £35:-…………..
TOTAL ENCLOSED: *£…………..

*(All cheques should be made payable to “British Society for Matrix Biology”)*

Please return registration form with payment to:
Dr Anne Vaughan-Thomas
University of Liverpool
Veterinary Science Building (Vet Annex)
Crown Street
Liverpool L69 7ZJ

For further information please contact Anne Vaughan-Thomas by telephone on +44 (0)151 794 4784 or email a.vaughan-thomas@liv.ac.uk
INSTRUCTIONS FOR SUBMISSION OF ABSTRACTS

BSMB Spring 2005 meeting, March 21st and 22nd 2005

COLLAGEN - FROM GENES TO FIBRILS
The Sherrington Buildings, University of Liverpool

Abstracts should be submitted by email and structured according to the format below using **Times New Roman font, text size 12-point as a MS-Word document.**
The text should fit within a box size of 16 cm x 26 cm. Abstracts not conforming to these specifications or with incomplete information may NOT be published.

**Information for Poster Presenters**
The deadline for submission of abstracts is 11th February

The poster boards will be **1.5 m high by 1 m wide.** Posters must be fixed with Velcro. Some Velcro will be available at the meeting, but please try and bring your own.

Please indicate whether you wish your abstract (i) to be submitted for the Poster Prize competition (presenter must be a PhD student member or be within their first three year post-doc appointment) and (ii) to be considered for an oral presentation (10 mins. + 5 mins. discussion).

Please ensure that you also complete a Registration form and return with your payment as detailed on the Registration Page.

**Title in bold**
**Authors in bold**
Affiliations

Introduction

Materials and Methods

Results

Discussion

**References**
Authors (Date) Title *Journal Name* vol. xx-xx

Submit abstracts as an email attachment to:
Dr Anne Vaughan-Thomas, a.vaughan-thomas@liv.ac.uk

Please indicate the name of **Corresponding Author, with fax and telephone numbers.**