

Connective Issues

BSMB Newsletter

British Society for Matrix Biology

Committee: Prof. Tim Hardingham (Chairman), Dr. Rose Maciewicz (Secretary), Dr. Jay Dudhia (Treasurer),
Dr. Jo Lewthwaite, Dr. Ian Clark, Dr. Anthony Day, Dr. Alison Reith, Dr. Norman McKie, Dr. Anthony Hollander

Registered charity no.
281399

No. 57 July 2000

Contents

- 2 Editorial *by Rose Maciewicz*
2 Current BSMB committee *contact information*
2 Publication of Meeting Abstracts in IJEP
2 Autumn Meeting 2000 in Newcastle *Register now*

Awards and Competitions

- 3 Bursaries for Autumn 2000 BSMB meeting in Newcastle *applications by August 14th 2000*

Forthcoming meetings

- 3 Spring 2001 BSMB meeting in Manchester *preliminary information*
3 Autumn 2001 BSMB meeting in Norwich *preliminary information*

Reports of previous meeting

- 3 Report of Spring 2000 BSMB meeting in London *by Jan Olaf Stracke, Marie Claire Hall and David Mahoney*

Appended

- Minutes of BSMB AGM held March 3rd 2000 in London
BSMB Annual Report and Accounts for Year ending December 1999
Programme for Autumn BSMB meeting Newcastle, September 11th - 12th
Notes, Directions and Registration form for Newcastle 2000 BSMB meeting
Bursary application form for Newcastle BSMB meeting

Urgent Request to BSMB Members

Would all members who have an email address please send it electronically to Rose.Maciewicz@astrazeneca.com along with your full name. We ask you to do this even if you have provided it previously as we wish to compile as complete a list as possible (error free and up-to-date). This will allow us to notify members more quickly of up-coming events and send you Connective Issues as an attachment ahead of the hard copy (or instead of it if you wish!). Greater use of email will help the Society keep costs down and maintain the membership fee at its present incredibly low level.

So please email the information NOW – it will only take a minute!

Editorial by Rose Maciewicz

Welcome to the 57th edition of Connective Issues.

First of all I am pleased to announce that the BSMB has been successful in its bid to hold the XVIIIth FECTS in the UK. The meeting will take place on 27th - 31st of July 2002 at the Brighton Centre.

Other Items for your attention include:

- Minutes of the recent AGM held in London.
- Registration form for the Autumn BSMB meeting at Newcastle.

Please make a note of the date for the Spring 2001 BSMB meeting which is ??????

Congratulations to Dr. Roger Smith, Royal Veterinary College, London who won the Veterinary Investigator Award sponsored by the Charity -Home of Rest of Horses at the BSMB Spring 2000 meeting in London. In addition congratulations to Emma Blain (Connective Tissue Biology Laboratories, Cardiff) and Mireille Vankemmelbeke (Dept. of Human Metabolism and Clinical Biochemistry, Sheffield) who won the IJEP Poster Competition at the meeting. Bursary recipients included: David Mahoney (MRC Immunochemistry Unit, Oxford); Marie-Claire Hall (School of Biological Sciences, UEA, Norwich); J.Anderson-Mackenzie (Collagen Research Group, Langford, Bristol); Jan Olaf Stracke (School of Biological Sciences, UEA, Norwich). Many thanks to Jan, Marie-Claire and David for writing the meeting report which can be found on pages 3-6.

FECTS 2000 bursary recipients included: Sally Dickinson (Dept. of Human Metabolism and Clinical Biochemistry, Sheffield); Steven Fenwick (Rheumatology Research Unit, Addenbrookes Hospital, Cambridge); Clare Curtis (Connective Tissue Biology Laboratories, Cardiff); Debbie Tudor (Connective Tissue Biology Laboratories, Cardiff); Gail Skinner (School of Biological Sciences, Manchester); and Emma Blain (Connective Tissue Biology Laboratories, Cardiff).

Note to check our website which can be accessed via <http://www.bsmb.ac.uk>. Between Newsletters all new information received by the Society can be found at the site.

See you all at Autumn 2000 meeting of the BSMB on September 11th & 12th at the University of Newcastle-upon-Tyne.

Current BSMB Committee

Officers:

Chairman, Prof. Tim Hardingham (University of Manchester; tharding@fs1.scg.man.ac.uk)
Honorary Treasurer, Dr. Jay Dudhia (The Royal Veterinary College, London; jdudhia@rvc.ac.uk)
Honorary Secretary Dr. Rose Maciewicz (AstraZeneca Pharmaceuticals; rose.maciewicz@astrazeneca.com)

Elected Members:

Dr. Jo Lewthwaite (Eastman Dental Institute, UCL; J.Lewthwaite@eastman.ucl.ac.uk)
Dr. Ian Clark (University of East Anglia; i.clark@uea.ac.uk)
Dr. Anthony Day (University of Oxford; ajday@bioch.ox.ac.uk)
Dr. Alison Reith (University of Bergen, Norway; alison.reith@pki.uib.no)
Dr. Norman McKie (Medical School, University Newcastle-upon-Tyne; nmckie@hgmp.mrc.ac.uk)
Dr. Anthony Hollander (University of Sheffield Medical School; a.hollander@sheffield.ac.uk)

Publications of BSMB Meeting Abstracts in International Journal of Experimental Pathology

The abstracts for the Spring 1999 meeting held in Oxford March 31st to April 2nd 1999 on 'The Molecular and Cell Biology of Wound Healing' were published in Int. J. Exp. Path. (2000) **81(1):A1-A29**.

The abstracts for the Autumn 1999 meeting held in Aberdeen on September 6th - 7th 1999 on "Biology and Therapeutic Strategies in Skeletal Disease" are to be published in Int. J. Exp. Path. (2000) **81(3)**.

The abstracts for the Spring 2000 meeting held recently in London on April 3rd and 4th on "Molecular Biology of the Synovial Joint" should be published latter this year.

Autumn 2000 BSMB meeting in Newcastle-upon-Tyne

The Autumn 2000 meeting of the BSMB will take place on the 11th - 12th September 2000 at the University of Newcastle-upon-Tyne. The topic is '**Cell-Cell and Cell Matrix Interactions in Development and Pathology**'. Registration forms are attached but additional copies can be obtained from the BSMB website or visit www.bsmb2000.ncl.ac.uk

The conference dinner will be held on the evening of Monday 11th September at the new International Centre for Life in the centre of Newcastle (see <http://www.centreforlife.co.uk> for further information). Some of the centre's entertainment sections will be available for the delegates, post dinner.

BSMB Bursary for Newcastle meeting

We are offering BSMB bursaries to attend the Autumn 2000 BSMB meeting in Newcastle. Young members of the Society are encouraged to apply for bursaries (up to a maximum of £100 for the BSMB) to assist with attending this meeting. An application form is included with this newsletter. Bursaries will only be considered if they are submitted on a current BSMB Bursary application form. Applications should be sent to the Secretary and not to the meeting organiser. **The application should be accompanied by a copy of the abstract to be presented at the meeting and a one page *curriculum vitae*.**

The deadline for receipt of bursaries to attend either meeting is Monday August 14th 2000.

The applications will be reviewed rapidly by the Committee and applicants will be informed of the outcome on or around 28th August 2000.

Criteria for Bursaries

1. Applicants should have been members of the Society for at least 1 full calendar year.
2. Applicants should be submitting an abstract and presenting a poster for the meeting to be attended.
3. Applicants should be at an early stage of their career (i.e. < 5 years from award of PhD) and unlikely to have access to travel funds. Most often where support for an overseas meeting is given this is the first such meeting they attend. For this reason emphasis is always given to young researchers who are generally in short term contract positions, i.e. mainly graduate students and occasionally early Post-docs. In addition the Committee will also take into account whether the applicant has received support from the BSMB within the last two years.
4. The work described in the abstract must be novel and likely to be of a quality that would reflect well as a BSMB supported contribution.

Spring 2001 BSMB meeting in Manchester

The Spring 2001 meeting of the BSMB will take place either on April 2nd / 3rd or 9th / 10th 2001. The venue for this meeting will be Hume Hall, University of Manchester and will be organised by Professor Tim Hardinham and Dr Cay Keilty.

This conference will have a main theme relating to connective tissue engineering. Speakers will cover various aspects of tissue engineering ranging from: delivery of growth factors and genes; biocompatibility; repair and regeneration of nerve, cartilage, blood vessels; Bioreactors; Designer proteins; and Bioengineering.

There will be a poster presentation session and several posters will be selected for short oral presentations during the main lecture series.

Preliminary Notification of Autumn 2001 BSMB meeting in Norwich

The Autumn 2001 meeting of the BSMB will take place in Norwich provisionally on September 3rd - 4th 2001. The topic will be "Impact of Proteases in Matrix Biology" covering diverse areas as: adhesions/motility; receptor shedding; protease and apoptosis; use of animal models and knockouts;. The organiser will be Dr. Ian Clark.

BSMB Meeting Report London, April 2000

'Molecular Cell Biology of the Synovial Joint' by Jan Olaf Stracke, Marie Claire Hall and David Mahoney

The spring meeting of the BSMB was held at the Royal Veterinary College University of London on 3-4th March and had as its theme Molecular Cell Biology of the Synovial Joint. The meeting was organised by Prof. Mike Bayliss and Dr. Jayesh Dudhia (The Royal Veterinary College, University of London) and attracted 160 participants. Financial support was provided by the sponsors The Wellcome Trust, Home of Rest for Horses, The Pet Plan Charitable Trust, Vetoquinol, Intervet Int BV, Janssen Animal Health, Smith & Nephew, TCS Biologicals, Sigma, Merck BDH, Fischer Scientific, Thermoquest, Triple Red, Wolf & BioRad, R & D Systems Europe Ltd and Life Technologies. Speakers were invited from research establishments in the UK, Germany, Canada and USA. The scientific session was divided into four sessions: (I & II) Bone and Cartilage, (III) Synovium and (IV) Tendon and Vasculature.

The meeting was opened by **Prof. Lance Lanyon** (London, UK) who talked on the responses of bone cells to biochemical and biomechanical stimuli. He proposed that post-menopausal osteoporosis can be viewed as a failure of bone's strain-related adaptation associated with the absence of estrogen. Results were described where the proliferation of primary osteoblasts and the osteoblastic cell line ROS 17/2.8, induced by strain or estrogen, was inhibited by the addition of estrogen antagonists. Using the cell line, he demonstrated that stable transfection of either, the estrogen receptor α (ER α) or the estrogen response element (ERE), increased the proliferative response to strain and estrogen. In both cases estrogen antagonists inhibited this proliferation. He showed, using specific inhibitors, that both stimuli act on the bone cell involving the MAPK pathway. He concluded that down-regulation of the pathway obviously shared by strain and estrogen could be the cause for the bone cells' reduced ability to proliferate and, thus, controlling the bone architecture when estrogen is absent following the menopause.

Prof. Klaus von der Mark (Erlangen, Germany) discussed the tightly regulated differentiation of chondrocytes during joint development and the reason why chondrocytes in the articular zone retain all features of the resting hyaline chondrocytes of embryonic cartilage anlagen. He highlighted unknown mechanisms which block further differentiation of these cells to hypertrophic chondrocytes and, finally, to bone-like or apoptotic cells. These control mechanisms are found to be out of balance during osteoarthritis, resulting in further differentiation to hypertrophic cells followed by matrix calcification and the ultimate loss of articular cartilage. He indicated that in studies using transgenic mice and cell culture systems, parathyroid hormone related peptide (PTHrP) and PTH were shown to prevent the transition of proliferating to hypertrophic chondrocytes, whereas thyroid hormones (T₃ or T₄) stimulate this differentiation. The identification of PTH- and T₃-responsive elements in the enhancer region of the human collagen type X gene, a molecular marker expressed by hypertrophic chondrocytes was also described. He concluded that characterisation of the cis- and trans-acting factors that are responsible for collagen type X expression may help to understand the mechanisms regulating hypertrophic differentiation.

This presentation was followed by three short selected poster presentations. **Dr. Vicki Church** (London, UK) discussed her work investigating Wnt signalling in chick limb chondrogenesis. She described distinct expression of various Wnt genes, along with one of their antagonists, in the developing chick limb using *in situ* hybridisation and proposed that Wnts provide candidate molecules for controlling the differentiation and development of cartilage in chicken. **Dr. K. M. Clements** (Bristol, UK) talked

about the influence of mechanical loading on the type II collagen denaturation in bovine cartilage-on-bone explants. Using an inhibition ELISA and an antibody against denatured collagen type II, he showed that cyclic loading increased the amount of denatured collagen type II in the explant cartilage. It was suggested that matrix degradation may occur as a direct result of severe mechanical strain rather than as a consequence of cell-mediated proteolysis. **Dr. Gillian Davies** (Cardiff, UK) presented results obtained from a chondrocyte pellet culture system. She demonstrated that cytokines, namely interleukin-1 (IL-1) and oncostatin M (OSM) not only up-regulate degradative enzymes (e.g. matrix metalloproteinases (MMPs)) but also alter the chondrocyte phenotype resulting in the production of a structurally modified matrix. It was concluded that chondrocytes may not be able to repair the damaged cartilage matrix during arthritic disease because of the cytokines present during these conditions.

Session II commenced with **Dr. Peter Roughley** (Montreal, Canada) who spoke about age-related changes in the biochemistry of articular cartilage proteoglycans. Two distinct groups of proteoglycans can be identified in this tissue: aggrecan, a hyaluronan binding proteoglycan (PG) and leucine-rich PGs that can interact with collagen, like decorin, biglycan, fibromodulin and lumican. Abundance and structure of both PG groups exhibit age-related changes due to changes in gene expression or proteolytic modification. In the case of aggrecan, increasing age is associated with decreased gene expression and increased proteolysis, whereas the leucine-rich repeat PGs appear more resistant to age-related hydrolysis. The enzymes responsible for the proteolytic processing were identified as MMPs and aggrecanases. Under catabolic conditions induced by IL-1, neither decorin, biglycan nor lumican were modified or lost from the matrix, whereas aggrecan was rapidly degraded and lost from the tissue. This depletion of aggrecan was due to aggrecanase cleavage and hyaluronan depolymerisation.

Dr. Roger Smith (London, UK) described the influence of loading, exercise and aging on equine tendon matrix. The study focused on changes in the levels of the cartilage oligomeric matrix protein (COMP), which is implicated in the organisation of collagenous matrix in load bearing tissues such as cartilage, ligament and tendon. It was concluded that tendon is able to adapt to biomechanical stimuli only during development, whereas it has a limited ability to adapt and repair after reaching skeletal maturity. Therefore, he proposed that high strain rates (training and racing) can produce cumulative fatigue damage of the equine tendon matrix, which can not be repaired, which results in clinical tendinitis. It was suggested that controlled exercise regimes of foals during skeletal development may help to prevent tendinitis in adult horses.

Prof. Roger Mason (London, UK) opened the third session on the Synovium. The synovium, which lines the synovial joint cavity, acts as a lubricator and protector of normal joint function at high pressures. The synovial interstitial matrix was described as a semi-permeable sieve, which allowed water and albumin to pass out whilst retaining the hyaluronan component. Production and accumulation of high molecular weight hyaluronan at the synovial surface correlated well with pressure increases which suggested that 2000kDA HA may have a role in opposing the osmotic loss of fluid from the joint. Rabbit joints were infused with either rooster HA, anionic or neutral dextran or Ringer vehicle. The results showed that in joints infused with 3-4mg/ml HA the trans-synovial drainage rate plateaued at $4\mu\text{l min}^{-1}$ where the intra-articular pressure was $>10\text{cm H}_2\text{O}$. This plateau effect showed that HA had a buffering capacity in opposition to synovial outflow in response to increasing pressure. With the control there was no plateau effect and opposition to outflow was reduced at high pressures. Neutral dextran gave no opposition to outflow with increasing pressure whereas anionic dextran of a similar molecular weight did reduce outflow. The pressure:flow relationship was shown to depend on polymer domain volume and charge rather than molecular weight *per se*.

Prof. Joe Edwards (London, UK) addressed the question of where synovial cells had evolved from. He reported the possibility of an evolutionary relationship between the cells of the synovial lining and of the bone marrow. Intimal fibroblasts found in the synovium differ from connective tissue fibroblasts in gene expression and in the matrix proteins they deposit. The synovial fibroblasts express decay-accelerating factor (DAF) and vascular cell adhesion molecule (VCAM) which are also expressed by nurse cells of the bone marrow. VCAM can be induced by TNF- α and IL-4 in the synovial fibroblasts, but not in dermal fibroblasts. Laminin, fibronectin, collagen IV, V and VIII are deposited by intimal fibroblasts. A comparison was drawn between the subpopulations of stromal cells in the bone marrow and the specialisation and segregation of the different fibroblast subpopulations in the synovium. It was proposed that the bone marrow may have "borrowed" the biology of the synovium which started off in the coelom of the simple invertebrates.

Four selected poster presentations were also presented in this session. **Dr. M. N. Vankemmelbeke** (Sheffield, UK) described the gene expression, localisation and activity of aggrecanase-1 and -2 in both human arthritic synovium and bovine synovium. Aggrecanases in cartilage were shown to be upregulated in response to hrIL-1 and all-trans-retinoate (Ret). The data presented suggested that aggrecanases -1 and -2 in bovine synovium were expressed constitutively and were not transcriptionally regulated by IL-1 or Ret. Aggrecanase-2 was also identified in human

arthritic synovium. The aggrecanase activity generated in the synovium cleaved aggrecan into fragments similar to those of chondrocyte-derived aggrecanases. It was concluded that the synovium-aggrecanases have a similar specificity to the chondrocyte aggrecanases and that aggrecanases in synovium may reach the cartilage, leading to the loss of cartilage aggrecan which occurs during arthritis.

Dr. J. Bowyer (Macclesfield, UK) proposed a Guinea pig model for osteoarthritic research. A 2-D magnetic resonance imaging system was used to image intact left-legs from osteo-arthritic Dunkin-Hartley 3-12 month old guinea pigs which were then examined histologically. Cartilage was removed from the right leg and cultured. The media was collected and MMP13 protein expression was detected by western analysis. Three month old Guinea pigs showed no changes which could be attributed to a progressive arthropathy. 12 month old guinea pigs, showed histologically detectable cartilage degeneration affecting the tibia, femur and meniscus. Focal cartilage thickening, loss or clustering of chondrocytes and degradation of the matrix was also observed. An increase in MMP13 protein was observed in places where cartilage lesions were detected. The imaging data highlighted where cartilage lesions had formed and complimented the histological data and the increase in active MMP13 detected.

Dr. J. M. Miotla (London, UK) reported vascular endothelial growth factor (VEGF) release associated with hypoxia in rheumatoid arthritis. Using CIA in a mouse model system, Oxygen levels and synovial perfusion rates were measured in the joints prior to or 10 days after the onset of arthritis. Synovial cells were isolated from these joints and VEGF mRNA levels measured. The $p\text{O}_2$ was shown to be significantly lower in the arthritic joints than in naïve or non-arthritic mice joints. The onset of arthritis was associated with expression of both VEGF mRNA and protein. It was concluded that a decrease in intra-articular $p\text{O}_2$ could act as a stimulus for VEGF production and the subsequent onset of arthritis. The perfusion rates of the synovium upon the onset of arthritis was not increased which suggested that angiogenesis associated with RA was not sufficient to restore O_2 homeostasis in the joint.

Dr. Helen Birch (London, UK) presented research into whether there are regional variations in equine superficial digital flexor tendons. Many injuries occur at the mid-metacarpal level and this region has the smallest cross-sectional area (CSA). The data showed little difference in collagen content between tendon regions, except that an increase in the metacarpo-phalangeal and phalangeal regions. The CSA was smallest in the mid-metacarpal region but was shown not to be the most susceptible to injury. Other factors such as hypoxia and/or hyperthermia maybe involved in tendon lesions.

Prof. Kathryn Vogel (University of New Mexico, Albuquerque, USA) opened the final session on tendon and vasculature with a detailed account of three different stages of mammalian tendon development using the bovine deep flexor tendon as an experimental model. Microscopy, antibody studies and in situ hybridisation were used to present a model of the tendon which is very heterogeneous, both within the adult tissue and during development from the embryo to the fetus. Differences in cell size, collagen type (I, II and IV) and proteoglycan expression (aggrecan, decorin, biglycan, COMP and fibromodulin) were shown between the tensional and compressed regions of the adult tendon. A major focus of this work was to discover how such differences in tendon arise. A probe for the transcription factor Scleraxix has shown this protein to be expressed during the earliest stages of tendon development in the embryo. This should allow the identification of genes crucial in very early tendon development.

Dr. Graham Riley (Addenbrooke's Hospital, Cambridge, UK) spoke of tendons as dynamic and metabolically active, and not as the inert structures once thought of. He presented work on the changes in cell populations and the extracellular matrix of patients with chronic tendon pathology. Differences in the expression levels of collagens, proteoglycans and non-collagen proteins were observed, probably mediated by changes in the activity of some members of the matrix metalloprotease family (e.g. increased activity of MMP-1). It was also suggested that cells from diseased and normal sites on the same tendon have differing responses to serum, cytokines and growth factors. Understanding how these disease responses are integrated with the normal functions by which the tendon responds to situations such as injury, mechanical strain and hypoxia, may offer new therapeutic targets.

Prof. David Blake (University of Bath, UK) presented the hypothesis that the flavoprotein xanthine oxidoreductase (XOR) may provide a bacteriocidal effect in the synovial environment by generation of superoxide and nitric oxide, as well as an inflammatory effect by generation of peroxynitrate. Both chondrocytes and osteoblasts containing XOR and antibodies against XOR have been found in many vascular membranes. Studies in breast milk have revealed that the pH and pO_2 optima for superoxide/nitric acid formation are those found under the hypoxic conditions of the synovial membrane, and antibodies against nitrotyrosine (the product of the reaction between tyrosine and nitric oxide) have been detected in the synovium. Prof. Blake suggested that whilst the anti-microbial properties of this system in the joint might be effective, it would lead to chronic inflammation. Hypoxia has already been shown to lead to the activation of genes resulting in tissue reorganisation and fibrosis and hence it is his belief that hypoxia is a suitable target for therapeutic manipulation in the prevention of synovitis and bone erosions.

Prof. Peter Winlove (University of Exeter, UK) discussed his work on measuring the rate of microvascular exchange between the bone-cartilage interface. Digital fluorescence microscopy was used to monitor the rate of exchange of fluorescently labelled probe molecules across the interface in the rat femoral head. Significant transport was demonstrated. The rate of uptake in intervertebral discs is also being investigated which they hope to relate with epoxy resin casts of the microcirculation. It was suggested that microchannel pores may exist in the interface. This is against conventional thinking and has implications for how cartilage may derive its nutrition, the feasibility of drug delivery systems etc.