

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, Prof. Anthony Hollander

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Editorial by Rose Maciewicz

Welcome to the 58th edition of Connective Issues.

Items for your attention include:

- Membership Renewal Information
- Programme and Registration form for the Spring BSMB meeting at Manchester
- Call for nominations for BSMB Committee Member
- Date for the Autumn 2001 BSMB meeting in Norwich, September 3rd - 4th.

Many thanks to Drs. N. McKie and M.A. Birch for the organisation of the Autumn 2000 BSMB meeting Newcastle upon Tyne. If you missed it a report of the meeting can be found in this newsletter. Thanks to Daniel Bax and Philippa Callender for writing it. Bursary recipients included: Dr. John Tarlton, Collagen Research, Bristol University; Mr. Daniel Bax, Wellcome Trust Centre for Cell-Matrix Research, University of Manchester; and Miss Philippa Callender, Connective Tissue Biology Group, Cardiff University.

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 Spring meeting in Manchester.

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; current e-mail ian.m.clark@astrazeneca.com)
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)
Prof. Anthony Hollander (University of Bristol Medical School; a.hollander@bristol.ac.uk)

Call for nominations for BSMB Committee Member

One committee member (JL) is due to retire in Spring 2001. We are therefore looking for nominations for this position. If you know of anyone who would like to be considered please write to the Secretary. Please note that this nomination must be seconded by another BSMB member. The nominated person must agree to the nomination and send a thumbnail sketch with

Name:

Position:

Research Interests:

Why I Want To Be On The Committee:

Nominations and thumbnail sketch must be received no later than February 28th 2001.

If required a postal ballot will be held prior to the AGM.

Publications of BSMB Meeting Abstracts in International Journal of Experimental Pathology

The abstracts for the Spring 2000 meeting held in London on April 3rd and 4th on "Molecular Biology of the Synovial Joint" are now published and can be found *Int. J. Exp. Path.* (2000) **81**:A1-A38.

The abstracts for the Autumn 2000 meeting of the BSMB which took place on the 11th - 12th September 2000 at the University of Newcastle-upon-Tyne on 'Cell-Cell and Cell Matrix Interactions in Development and Pathology' are expected to be published in February 2001.

Membership Renewal Information

The membership fees for 2001 are now due. We are requesting that all members complete the attached form '**Membership Subscription for 2001**', which can be found on the penultimate page of this newsletter. The completed form with membership payment should be forwarded to the BSMB Secretary. The form should be self-explanatory but if you have any queries please contact the Secretary by e-mail.

Note that the 2001 membership fee is £10.00 for full members and £2.00 for student members. As in the past no subscription is payable by Honorary Members.

If you pay by Direct Debit please confirm that your Bank has the correct details, which are as follows:
Account name: British Society for Matrix Biology
Account Number: 09670343
Sort Code: 60-00-01
Branch: NatWest,
City of London Office,
PO Box 12258,
1 Princess Street,
London EC2R 8PA

If you do not pay by Direct Debit please send us a cheque immediately. Please note that the membership fee is collected on January 1st of the year. If you fail to send us your 2001 subscription fee we will have to cancel your membership. Please help the Society to stay solvent by paying your membership fees.

Under the provision of the Data Protection Act 1994 s.33(3) we are required to inform our members that we are holding a mailing list on computer disc. The information is only for the purpose of distributing or recording the distribution of articles of information to members and consists only of their names; addresses and other particular necessary for such distribution. A member objecting to the information being held as mentioned should notify the current BSMB Secretary.

Announcement - Barbara Robert Memorial Medal to Professor John Scott

I am delighted to inform the BSMB membership that one of our members, Professor John Scott of University of Manchester, has been awarded the Barbara Robert Memorial Medal and citation by the Societe Francais du Tissu Conjonctif at their Annual Meeting in Rennes in 2000. This was the 25th year in which the award was made. Professor Scott gave a lecture entitled "Extracellular matrix, supramolecular organisation and shape". I am sure you will join me in congratulating Professor Scott on this award.

Spring 2001 BSMB meeting in Manchester

The Spring 2001 meeting of the BSMB will take place on April 2nd - 3rd 2001 and the topic is "Cellular and Molecular Mechanisms in Tissue Engineering". The venue for this meeting will be Hulme Hall, University of Manchester and will be organised by Professor Tim Hardingham and Dr. Cay Kielty. Registration forms are attached but additional copies can be obtained from the BSMB website.

The conference dinner will be held on the evening of Monday 2nd April at Manchester United Stadium, Old Trafford.

Annual General Meeting

The Annual General meeting will be held at the Spring BSMB meeting on 2nd April 2001. The preliminary agenda follows. Items for inclusion should be sent to the BSMB Chairman, Professor Tim Hardingham and arrive no later than March 5th 2001.

Preliminary AGENDA
BRITISH SOCIETY OF MATRIX BIOLOGY
ANNUAL GENERAL MEETING
2nd April 2001
17:15
Hulme Hall, University Manchester

1. Approval of Minutes of the last AGM held in London on April 3rd 2000 (Tim Hardingham)
2. Matters Arising (Tim Hardingham)
3. Secretary's report (Rose Maciewicz)
4. Treasurer's report (Jay Dudhia)
5. Election of New Committee Member (Tim Hardingham)
6. AOB

BSMB Bursary for Manchester meeting

We are offering BSMB bursaries to attend the Spring 2001 BSMB meeting in Manchester. 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 can be found on Society's website <http://www.bsmb.ac.uk>.

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 the meeting is Monday, February 19th 2001.

The applications will be reviewed rapidly by the Committee and applicants will be informed by email by Monday, February 26th.

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.

Support of IJEP Poster Competition

Win a prize for your poster

Will you be presenting a poster at the Spring BSMB meeting? It took hours to put together, you're presenting some good work and you are pleased with how your poster looks. THEN WHY NOT ENTER OUR NEXT POSTER COMPETITION AND WIN ONE HUNDRED POUNDS? The poster competition, which is sponsored by the International Journal of Experimental Pathology, is designed to reward PhD students and Post-docs, who put a lot of time and effort into making an excellent collection of posters at the BSMB meetings each year. If you are a PhD student or Post-doc please enter the competition. You have nothing to lose and you may just win one hundred pounds. The next competition will be held at the Spring BSMB meeting in Manchester. If you are a supervisor, encourage your PhD students and Post-docs to enter the competition. Not only will they receive some money, but more importantly it will draw attention to their work and receipt of the reward might be a useful addition to their CV!

IJEP Poster Competition Rules:

1. It will be held once per year and at a BSMB meeting selected by the Committee.
2. The competition will be publicised well in advance.
3. The competition is open to PhD students and recently qualified post-docs (up to 2 years), who must indicate prior to the meeting their intention to enter the competition.
4. Up to 3 scholarships of £100 each will be awarded, dependent on the quality of the presentations. If the quality is low, no awards will be made.
5. The posters will be judged by at least 3 Committee members who will view the posters as well as discuss the work with the poster presenter. Criteria for judging the posters will be: clarity of the presented poster; scientific content; and scientific understanding of the work.
6. The award to be used by the recipient as they choose.
7. The recipients of the award to be notified at the meeting and also to be listed in the next newsletter issue.

Preliminary Notification of Autumn 2001 BSMB meeting University of East Anglia, Norwich

"The Impact of Proteinases on Matrix Biology"

The autumn meeting of the BSMB will be held at the University of East Anglia (UEA), Norwich on Monday 3rd and Tuesday 4th September 2001. This meeting is being organised by Ian M. Clark, UEA, Norwich; current contact details are: Tel. 01625-519834, e-mail: Ian.M.Clark@astrazeneca.com. UEA is a campus based university, and residences, lecture theatre, poster venue and conference dinner are all close together.

The conference will address the role of proteinases in matrix biology and pathology. Speakers will discuss a wide variety of biological systems and technologies. Speakers who have currently accepted an invitation to speak include:

Voon Wee Yong, U. Calgary, Canada: *MMPs in the CNS - friend or foe?*

Drew Rowan, U. Newcastle, UK: *title tba*

John Iredale, U. Southampton, UK: *Recovery from liver fibrosis: matrix degradation and the fate of the hepatic stellate cell*

Shu Ye, U. Southampton, UK: *Polymorphisms in MMP and TIMP genes*

Vince Ellis, UEA, UK: *Mechanisms regulating plasmin generation in the pericellular environment*

Andy Baker, U. Glasgow, UK: *Death by TIMP-3: Insights into mechanisms and exploitation in gene therapy for disease*

Kevin Leco, U. Western Ontario, Canada: *Development of the mouse lung in the absence of TIMP-3*

There will be poster presentations, and posters will be selected for short oral presentations during the main lecture series. Deadlines for abstracts will be in the 1st week of August 2001, and registration forms will be available late June 2001.

There will be a conference dinner on the Monday evening in the Sainsbury Centre for Visual Arts and the chance to view the Robert and Lisa Sainsbury art collection. This is reputed to be one of the most stimulating collections formed in Europe during the 20th century, it combines modern Western art with fine and applied arts from Africa, the Pacific, the Americas, Asia, Egypt, medieval Europe and the ancient Mediterranean. The Sainsbury Collection is particularly well known for its holdings of Francis Bacon, John Davies, Alberto Giacometti and Henry Moore.

XVIIIth FECTS

The XVIIIth FECTS will be hosted by the BSMB in the UK and take place on July 27th - 31st 2002 at the Brighton Centre. If anyone would like to help with the organisation please contact the Secretary.

WORKSHOP ON FIBROUS PROTEINS

A workshop on 'COILED-COILS, COLLAGEN & CO-PROTEINS: III' will be held at the BÖGLERHOF HOTEL, in ALPBACH, AUSTRIA on September 16-21, 2001. The organisers are David Parry, Robert Goldman, John Squire. The Workshop will be run on the same lines as the last of these Workshops in 1997. The organisers hope to provide bursaries for younger scientists. For further details and a registration form contact: a.baruwa@ic.ac.uk

Sessions include:

Coiled-coil structures (Chair: Ueli Aebi)

Intermediate Filaments (Chairs: Peter Steinert, David Parry)

Intermediate Filament Associated Proteins (Chair: Robert Goldman)

Muscle Proteins (Chair: John Squire)

Microtubules and Motors (Chair: Eckhard Mandelkow)

Hot Topics (Chair: Alasdair Steven)

Collagen Molecular Structure (Chair: Andrew Miller)

Connective tissue (Chair: Darwin Prockop)

Special Lecturer: Juergen Engel

BSMB Meeting Report

Autumn 2000 BSMB meeting in

Newcastle-upon-Tyne

'Cell-Cell and Cell Matrix Interactions in Development and Pathology'

by Philippa Callender and Dan Bax

The autumn meeting of the BSMB was held at the University of Newcastle-upon-Tyne on the 10th-11th September and had as its theme Cell-cell/cell-matrix interactions in connective tissue. The meeting was organised by Dr Norman McKie and Dr Mark Birch and attracted 93 delegates. Financial support was provided by the sponsors Carl Zeiss Ltd., Roche, Wyeth-Ayerst Research and Pfizer Ltd.

Prof. Cheryl Tickle (Dundee, UK) opened the meeting with an overview of the molecular basis of vertebrate limb development. The chick embryo was used to investigate the complex arrangement of cells, their matrix and their interactions within the limb bud. Three axes were described, showing different interactions within the developing limb. Proximal distal recognition was determined by a fibroblast growth factor (FGF) signalling loop between the mesenchyme and the apical ectodermal ridge (AER) which conferred outgrowth of the limb bud. Removal of the AER revealed a truncated limb and addition of FGF resulted in continued outgrowth. Specific expression of the Wnt7a gene in the dorsal ectoderm ensured the orientation of the limb bud in a ventral and dorsal pattern. The functional inactivation of Wnt7a caused a double ventral phenotype, showing it was active only in the dorsal part of the limb. Digit development was shown to be determined in the polarising region of the

limb bud on the anterior-posterior axis. Retinoic acid (RA), Sonic hedgehog (Shh) and bone morphogenic protein (BMP-2 and 4) co-operate with FGF from the mesenchyme to activate the HOX genes (9-13) which pattern in digit development. Grafting of the polarising region to the anterior margin resulted in mirrored patterning of digits. This suggested that the polarising region produces a morphogen in a graded long range signal which acts in a dose dependant manner to give the pattern required for correct limb development. Addition of Shh to the anterior margin resulted in the formation of an extra thumb and addition of BMP-2, at regular time points, resulted in the formation of specific digits. The inhibition of BMP-2 resulted in identical digit formation with no patterning. BMP-2 is the morphogen required for digit formation but it cannot function without a gradient of Shh, which acts as a competence factor. This local signalling capacity of Shh was shown to be due to a variation in the expression of its receptor, Patched (Ptc), which on binding makes cells competent for BMP-2 signalling resulting in digit formation.

Prof. Anthony Hollander (Bristol, UK) discussed new strategies for the prevention and repair of cartilage in arthritis. He described a new method for the treatment of small lesions where a hyaline cartilage tissue, constructed by chondrocytes on a polyglycolic acid (PGA) scaffold, could be grafted into the lesion and integrated into the surrounding tissue. Tissue formation was achieved by slowly rotating the isolated chondrocytes in a spinner flask containing the PGA scaffold for one week with bFGF, followed by insulin and ascorbate, to instigate cell differentiation and matrix production. The source of the chondrocytes was found to be critical for the constitution of the tissue produced. Articular cartilage, with a heterogeneous population of cells, may not lay down the hyaline cartilage expected, whereas nasal cartilage containing a homogeneous population of chondrocytes was considered as a better source of cells. He showed data where the type II collagen content of normal hyaline cartilage was significantly greater than that of the engineered cartilage. Chondrocytes derived from articular calf cartilage adhered to the outside of the PGA scaffold produce a cartilage matrix, but a lack of cells internally resulted in no matrix deposition within the scaffold making the tissue weak. In comparison, nasal chondrocytes were better at migrating into the scaffold and consequently had a more uniform distribution of type II collagen. The levels of type II collagen and proteoglycan were measured in nasal and articular, human and bovine cartilages engineered on the scaffold. The results showed that nasal cartilage produced larger amounts of both proteoglycan and type II collagen and that bovine chondrocytes were considerably better than human cells, producing a more substantial matrix. The overall conclusions were: that nasal cartilage is a better source of chondrocytes for tissue engineering than articular cartilage; engineered cartilage has a low type II collagen concentration and has a slow synthetic rate; the organisation of engineered cartilage matrix into discrete zones has not yet been achieved.

Prof. Michael Horton (UCL) gave a comprehensive talk on bone resorption and its role in osteoporosis. Whilst the recently discovered RANK/RANKL system represents a mechanism for osteoblast and osteoclast coupling, signals that cause fusion of mononuclear precursors, attachment of osteoclasts, retraction of osteoblasts and removal of osteoid are unknown. A mechanism for bone resorption by the osteoclast, which is the route thought to cause the loss of trabecular structure and bone mass seen in osteoporosis was reported. The initial stage was the adhesion of the osteoclast to the cell matrix, followed by the dissolution of mineral and the degradation of the matrix. At the site of resorption, the osteoclast has a ruffled border that forms a tight sealing zone, causing a low pH, resulting in the dissolution of mineral. The degradation of the surrounding matrix is achieved by MMPs and lysosomal cysteine

proteases, including cathepsin K, which is restricted to osteoclasts and is therefore likely to have a specific role in bone resorption. Mutations in cathepsin K cause an osteopetrotic phenotype. The degradation products are processed through the osteoclast by transcytosis. The initial adherence and recognition of osteoclasts to the matrix is still poorly understood. Prof. Horton discussed how the search for potential osteoclast attachment molecules identified high levels of the receptor, $\alpha_5\beta_3$ integrin, as well as lower levels of $\alpha_2\beta_1$ and $\alpha_5\beta_1$ integrins. The $\alpha_5\beta_3$ integrin was shown, by an in vitro model system, to mediate the interaction of osteoclasts with both fibronectin and vitronectin through the RGD sequence. Echistatin was used as a blocker of RGD binding and resulted in the retraction of cells and a loss of the cell spreading required for bone resorption. The production of RGD analogue peptides that interfere with osteoclast attachment may therefore inhibit bone resorption, show such molecules may inhibit the progression of osteoporosis and thus represent good targets for a potential therapy.

Dr. John Tarlton (Bristol, UK) presented data from his work on molecular mediators of matrix metabolism and their effect on wound healing in chronic dermal ulcers. The levels of proMMP2, proMMP-9, TIMPS 1 and 2, and type I collagen propeptide (PICP) in acute wounds (control) and venous ulcers were compared. The results demonstrated that the co-ordination of these molecular elements detected in acute wound healing are lost in chronic ulcers. This disruption in matrix metabolism may be due to a loss of feedback regulation and could have implications in the future treatment of chronic dermal ulcers.

Dr. Paul Koshy (Newcastle, UK) discussed his work investigating the ability of IL-17 to promote cartilage collagen breakdown, both alone and in combination with other proinflammatory cytokines (IL-1 β or IL-6). This study on bovine nasal cartilage explants showed that IL-17 alone induced collagen breakdown and increased collagenase activity. IL-17 combined with IL-1 revealed a synergistic effect on collagen release. Both oncostatin M and IL-6 operated synergistically with IL-17 to induce a significant collagen loss. It was concluded that IL-17 induced chondrocyte-mediated, MMP-dependent collagen breakdown in cartilage and acts synergistically with IL-1, IL-6 and OSM to further induce the degradation of cartilage collagen.

Prof. Christopher Day (Newcastle, UK) talked on the role of hepatic stellate cells (HSC) and their activation in liver fibrosis. HSC provide the liver with its extracellular matrix. An imbalance in the turnover of the ECM results in an increase in both the interstitial collagens and the collagen I:III ratio characteristic of fibrosis. HSC respond to injury and disease with a change of phenotype to become 'activated' HSC. Factors that initiate the activation of HSC include tumour necrosis factor (TNF)- α , transforming growth factor (TGF)- β , endothelin and oxidative stress. The role of p38 mitogen-activated protein (MAP) kinase and c-jun N-terminal kinase (JNK) in HSC activation was discussed, since these have been reported to exist downstream of the initiating factors in other cells types. The results showed that p38 MAP kinase and JNK were activated by TNF- α and endothelin, suggesting that these signalling pathways are present in HSC activation. The effects of TNF- α were inhibited by the antioxidant 2-mercaptoethanol showing that this is a stress activated response. The activity of p38 MAP kinase was higher in transformed cells than in quiescent cells and was reduced by a specific inhibitor, providing evidence of a role for p38 MAP kinase in HSC activation. A second pathway was also discussed. The potent mitogen, platelet-derived growth factor (PDGF) sustains the production of phosphatidic acid (PA), a lipid-derived second messenger, that stimulates HSC proliferation and increases the activity of

the extracellular signal regulated kinase (ERK). HSC proliferation was completely blocked by the inhibition of ERK activation highlighting the importance of this signalling molecule in this pathway. Results of a comparison between PDGF and TGF- β suggested that the potent mitogenic effect of PDGF in HSCs may be caused by the production of PA and subsequently by a more sustained activation of ERK than occurs with less potent mitogens that do not generate the second messenger PA.

Dr. Christopher Little (University of Cardiff, Wales) summarised work on the role of aggrecan metabolism in the progression of articular cartilage degeneration. In the early stages of cartilage degeneration there is little collagen loss, but large amounts of aggrecan loss; by the latter stages there is a large amount of collagen release and little aggrecan release. Antibodies generated against the MMP and aggrecan cleavage sites of aggrecan were used to show that aggrecanases were responsible for the early phase of aggrecan release, while latter release of aggrecan was via MMPs. Further aggrecan cleavage was observed within the C-terminal region, releasing the GAG bearing fragment of aggrecan. This was present in human and bovine cartilage and was reduced in the presence of MMP inhibitors.

Dr. David Young (U.E.A) gave a talk describing the elements within exon 1 and intron 1 involved with the transcriptional control of the TIMP-1 gene. The start of the TIMP-1 reading frame is within exon 2, so exon 1 and intron 1 are untranslated. Deletion mutants fused to the CAT reporter system showed that the TIMP-1 promoter region +28 to +60 acted positively. This element was position dependent and sequence inversion gave only partial activity. It contained the 5' splice site and half sites for the binding of Leader Binding Protein-1. The sequence +22 to +58 was shown, by south western blotting, to bind to a mystery factor A, but not LBP-1

Ms. Farinaz Afsari (University of Edinburgh) gave a description of the wingless (*wnt*) signaling pathway initiated when *wnt* interacts with *Fz* receptors on the cell surface. Previous studies have shown that the specificity of this interaction is increased by the presence of GAGs. Immunofluorescence data was displayed showing that *wnt* co-localises with *Fz*, fibronectin and CD44 at zones of cell-cell interaction, indicating that fibronectin or CD44 could function as the GAG co-receptors.

Mr. Daniel Bax (University of Manchester) gave a presentation on integrin binding properties of TNF- α converting enzyme (TACE). As a member of the ADAM family of proteins, TACE contains a number of extracellular domains including a Pro, metalloprotease and disintegrin domain. TACE constructs containing the whole extracellular domain, the metalloprotease domain, or the disintegrin domain were expressed as Fc fusions. The whole extracellular and disintegrin domain constructs were capable of supporting skin fibroblast cell spreading, while the Metalloprotease domain was not. Anti-integrin monoclonal antibodies showed that cell spreading was integrin $\alpha_5\beta_1$ dependant. This was supported by purified integrin $\alpha_5\beta_1$ association with TACE.

Dr. Donald Salter (University of Edinburgh) elucidated the mechanotransduction receptors and pathways activated in mechanically stimulated chondrocytes. Mechanical stress was simulated by seeding cells onto a plate flexed at 33Hz. The mechanical stimulation caused hyperpolarisation of the normal chondrocyte plasma membrane, which was dependent on the ECM to which the cells were adhering. Anti-integrin monoclonal antibodies showed a dependence on integrin $\alpha_5\beta_1$ and inhibition of integrin signaling molecules gave a reduction in hyperpolarisation. The hyperpolarisation effect was found to be autocrine in nature, with media taken from

stimulated cells eliciting the effect on non-stimulated cells. The factor involved was IL-4, and IL-4 KO cells did not respond to mechanical stress. Down stream effect of hyperpolarisation was a reduction of MMP-3 and aggrecan synthesis. In contrast to normal chondrocytes, osteoarthritic chondrocytes displayed a depolarization of their membranes under mechanical stress. Stretch activated channels and tyrosine kinases were involved in this response, whereas the cytoskeleton and integrin $\alpha_5\beta_1$ was not. These cells had little sensitivity to IL-4, but IL-1B reduced the depolarization response. Mechanical stress did not have the effects on MMP-3 and aggrecan levels observed in normal cells.

Prof. David Becher (Galaxo Wellcome U.S.A.) described the properties of the metalloprotease activity of TNF α converting enzyme (TACE). TNF α processing from the cell surface at the Ala76-Val77 bond was due to an activity associated with the cell surface which was TIMP insensitive. This was cloned and found to be a member of the ADAM family of proteins. TACE KO mice had a 90% reduction in TNF α cleavage, the remaining cleavage was due to ADAM-10 which is 30% identical to TACE. Compounds blocking TACE activity reduced the swelling and pain induced by PGPS injection into mice joints. In human clinical trials these compounds reduced TNF α spiked 1-2 hours after LPS injection into the joint. Candidate and random approaches were used to find substrates for other ADAM proteins. ADAM-9 was found to cleave APP, TNF α and KL-1, but not at the physiological site. Finally a phage display system was used to find novel cleavage sites recognized by metalloproteases. This identified that aggrecan and types II and IV collagen are potential substrates for collagenase III.

Prof. Tim Skerry (York University) revealed a role in bone formation for the glutamate transporter whose function is well documented for the central nervous system. Loading of rat forearms resulted in bone formation on the outside surfaces of the ulnar. Differential display on these sections suggested that the glutamate transporter might be functioning in response to load. A NMDA antagonist, MK801, reduced osteoblast function in bone. Through the control that these cells have on the bone reabsorbing osteoclasts, the drug was found to reduce osteoclast function, and so reduce bone re-absorption. This effect was only observed if the drug was administered at an early stage, so is probably involved in osteoclast differentiation rather than cellular function. The NMDA receptor functions through cFos expression and was found to co-localize with synaptic marker synapsin I at the cell junctions. This indicates the presence of synapse like structures in bone cells.

Prof. Hermona Soreq (Hebrew University, Jerusalem, Israel) discussed the role of acetyl cholinesterase in neurological disturbances caused by anti-chemical warfare drugs and the stress response. Under conditions of stress the blood-brain barrier was more permeable. This correlated to an increased blood pressure and body temperature. Cloning of the acetyl cholinesterase gene gave two clones, one from the liver and the other from the brain. Mutations in both were present in soldiers with sensitivity to the anti-chemical warfare drugs. The amounts of each component of the stress response pathway, cFos, cholinacetyltransferase, acetyl cholinesterase and syntaxin all changed with the result of dampening down the stress response under conditions of stress. Acetyl cholinesterase is the universal stress response being up regulated in many different forms of stress. Transgenic mice with up-regulated levels of acetyl cholinesterase were more sensitive to chemical stress. They had abnormal neuro-muscular junctions, and antisense to acetyl cholinesterase reverted this to normal muscle function. Acetyl cholinesterase was shown to be capable of inducing neurite sprouting. This was not dependent on the

catalytic activity, but was due to the C terminus of the protein.

XVIIth Meeting Report of Plenary Lectures Patras Greece by Steven Fenwick

At the opening ceremony on July 1st, Dr. H. Ahrweiler presented us with the fact that we are all responsible for the ethics of science. Although it is not unethical to be wrong, it is unethical to be fraudulent. Thus the correct portrayal of everything that we do is imperative. In fact, all history should be portrayed accurately, be it scientific or otherwise. Education of the young is vital, ethically they should be presented with an exact depiction of the facts. Examples such as the Human Genome Project show how economic interest may jeopardise ethical issues, and the setting up of a Bioethics committee was addressed to deal with such issues.

Dr. Jurgen Engel discussed general features of oligomerisation domains, such as coiled coils and triple helices and how they form higher structures. He then went on to describe the triple chain structures of laminin, and the pentameric chain structure of COMP. Of most interest in the COMP molecule is the presence of a hydrophobic channel that was theorised might be loaded with a hydrophobic molecule in tendon and/or cartilage. The biochemistry of coiled coils was alluded to and some of the structure and functions of a number of proteins containing coiled coils discussed, including matrilin-1, phospholamban and foldon.

Dr. Karl Kadler posed the question as to how cells only microns in size make collagen fibrils that can be up to millimetres in length. The solution was shown to be the fusion of smaller fibrils. Cells were shown to produce the smaller fibrils, which are then packaged into larger assemblies intracellularly. The binding of non-collagenous macromolecules was also described. 3D EM tomography was developed, and used to visualise the microfibril structure of corneal collagen allowing us further insight into how collagen molecules form larger structures.

Dr. Alfonso Colombatti described the *in-vivo* and *in-vitro* expression of EMILIN-1 (Elastin Microfibril Interface Located Protein) and its localisation at the interface of elastic fibres and microfibrils. Human EMILIN-1 was cloned and shown to contain a C1q and coiled coil domain. The various structural domains of EMILIN-1 were described, along with their ability to form large aggregates. A second EMILIN, EMILIN-2 was also described and the possible associations between the two were shown. Finally, the various properties of EMILIN were shown, including its cell binding and pro-migratory activities.

Dr. Mats Paulsson asked how are proteoglycan aggregates associated with the collagen matrix, via attraction or interaction? He described the structure of cartilage matrix protein or matrilin-1, found on the core protein of aggrecan, and went on to describe matrilins-2, 3 and 4 and the assortment of oligomers that they can form. Differential immunolocalisation was shown in the mouse sternum and ribs, and in mouse cartilage, and further tissue specificity was shown, matrilin-2 localised in various basement membranes, matrilin-4 expressed in oesophagus as examples. Finally showed a co-distribution of matrilins and various other matrix molecules, and showed that matrilins have a high affinity for collagen via a von Willebrand Factor A-like domain.

Dr. John Gallagher informed us that heparan sulphate proteoglycans (HSPG) are the main cell surface proteoglycans on most cell types and acts as a co-receptor for growth factors and ECM molecules. The structure of HS was described and the importance of the sequence of sulphated domains emphasised in growth factor binding, regulation and cell behaviour. HSPGs bind growth factors and dependent on the sulphated sequence mediate structural changes to allow receptor binding. The biosynthesis of HS was described as highly complex, the enzymes (of which numerous isoforms exist) being responsible for defining the exact structure. Mutated HS that lack 2-O-sulphation showed alterations in the sulphation pattern that altered binding to various growth factors though activation of the factors was similar. Development in mutants appeared normal. Sulphation patterns may therefore be required for more refined regulatory properties of HS.

Dr. Lars-Ake Fransson summarised the differences in biosynthesis of decorin (a secretory product) and glypican (a membrane anchored product), and the recycling pathway of glypican. Experiments were described where the various stages of decorin and glypican biosynthesis were interrupted to allow study of intermediary molecules. The biosynthesis of decorin relies upon removal of a phosphate group from its xylose residue at the tri-saccharide (Xyl-Gal-Gal) stage, suggesting that the tri-saccharide acts as a polymerisation signal. Glypican can be arrested in its recycling process with various side chain lengths. NO was shown to be important in this as inhibitors of NO caused alterations in chain length.

Dr. Norbert Perrimon discussed the role of HSPG in ligand receptor interactions and summarised the biosynthesis of HSPG GAG chains, including the role of some of the individual enzymes involved. Further, a number of mutations in these enzymes were described. The role that a HSPG plays in the signalling of a number of proteins, including FGF, wingless and hedgehog was described in *Drosophila* development. Finally, the HSPG Dally-Like Protein (DLP) was described, and the various phenotypes associated with mutations in DLP were characterised.

Dr. Guido David discussed HSPGs associated with the cell surface and the many roles that they play, for instance cell-cell and cell-matrix interactions. The HS molecule was described as an instructive template and is linked to many proteins via modular structures, example given being the glypicans and syndecans. A specific protein domain, known as a PDZ domain was shown to have a groove that is thought to bind to protein. These domains were studied by using functional mutations and it was suggested that at least two PDZ domains were required for protein binding. Finally, a protein named syntenin was hypothesised to drive the assembly of syndecan, and its various domains and functions were described.

Dr. Klaus von der Mark informed us that the cell surface receptor $\alpha_7\beta_1$ is distributed mainly in muscle and mediates cell adhesion to laminin. A number of experiments, including knockout mice and antibody blocking, were described to show the binding of α_7 to laminin. The intracellular signalling of α_7 via its cytoplasmic domain was illustrated, but how this domain is linked to the cytoskeleton was questioned. A protein named 'plectin', which is almost ubiquitous, was shown to co-localise with α_7 in the RuGli cell line when plated on laminin, and further studies showed that plectin links α_7 to the actin-myosin filament system.

Professor Heikki Rauvula showed that two factors from fractionated rat brain extracts were responsible for neurite outgrowth, named amphoterin and HB-GAM. The structure, function and cell surface receptor (syndecan) of HB-GAM

were discussed, and its importance in osteoblast recruitment and bone maintenance illustrated by transgenic mice experiments. The ability of amphoterin to regulate cell motility was of prime concern, and a number of experiments were described that showed amphoterin to be a regulatory molecule in cell invasion/migration via binding to the RAGE cell surface receptor.

Dr. Hugh Lavery described an intriguing model of a TGF- β_3 knockout mouse, which forms a cleft palate due to the non-fusion of medial edge epithelial cells. It was shown that these palate shelves could be cultured *in-vitro* and the addition of exogenous TGF- β_3 allowed normal palate fusion. Cell migration is an important event in palate fusion. The results of *in-vitro* migration assays and foetal wounding experiments on TGF- β_3 knockout cells/mice and were shown along with data from a rat incisional model all implied the role of TGF- β_3 in improving wound healing. Finally, type VI collagen was suggested as an important factor in palate fusion. Gene array technology showed a down regulation of this molecule in TGF- β_3 knockout mice.

Professor Rupert Timpl informed us that laminins are the most important ligands for cellular receptors. Laminin G domain-like (LG) modules were described and their binding abilities tested by constructing mutant LG domains. The importance of specific sites required for binding other molecules was discussed, along with the similarities and differences between different LG domains. Finally, a hypothesis was put forward for the structure of how the complete laminin molecule would fit together.

Dr. Dick Heinegard asked how collagen fibril diameters within the cartilage matrix could be regulated by the cell, and how do different fibril diameters come to be. The structure and function of a number of leucine rich repeat proteins of the ECM were described e.g. decorin, biglycan. The proteins chondroadherin, COMP, fibromodulin and lumican were discussed in further detail. The structural details, including collagen binding sites and the mechanisms by which these proteins may regulate collagen fibril diameter were contemplated.

Professor Ryu-Ichiro Hata discussed the role of type I collagen in regulating the morphology and collagen synthesis of fibroblasts, and how the synthesis of type I collagen is regulated by the surrounding matrix of the cell. The gene sequence of type I collagen was shown to have 2 unique sequences. Various constructs of this gene showed that both sequences were essential for transcription. Microsatellite sequences in the gene were also discussed in terms of gene polymorphism, and how polymorphisms are related to disease susceptibility.

Dr. Benoit de Crombrughe discussed the steps involved in the differentiation of mesenchymal cells into chondrocytes, and some of the growth factors and cytokines involved in this sequence of events. This differentiation is controlled by specific transcription factors, of which Sox 9 is an important member. The structure and expression of Sox 9 was described and it is known to direct type II and XI collagen synthesis. Numerous experiments on Sox 9 knockouts and chimaeras were shown and homozygous negative genotype cells were excluded from cartilage at the mesenchymal condensation stage. The structure, function and localisation of two further transcription factors L-Sox 5 and Sox 6 were also described, along with experiments to determine their role in chondrogenesis.

Dr. Jyrki Heino gave a summary of the collagen binding integrins and described the basic $\alpha_1\beta_1$ receptor complex that requires an I domain to allow collagen binding. The α_1 and β_1 domains were described and the importance of Asp219

in collagen binding highlighted. Though different I domains are structurally similar, work was shown to suggest that there is specificity for certain collagen types. The phenotype of $\alpha_1(I)$ knockout mice was described and some experiments behind the elucidation of integrin signalling were described.

Dr. Michael Briggs described how mutations in structural components lead to various diseases. Two main examples discussed were pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED). PSACH results from mutations in COMP, while the mutation location is more varied in MED. The types of mutations seen in COMP were described, along with mutations seen in the COL9a2 allele of type IX collagen. Linkage analysis of a family with MED was discussed, up to 60% of the mutations were unknown, the rest being in COMP and collagen IX. Finally it was shown that if COMP is mutated, it is not secreted, which might explain the disruption of articular cartilage in these diseases.

Dr. Thomas Kreig suggested that fibroblast monolayers and collagen lattice cultures are relevant models for study and gave examples of how fibroblasts in these models show similar response to cells *in-vivo*. The effects of interactions with the matrix on metalloproteinase and collagen synthesis were described. Systemic scleroderma fibroblasts were shown to behave differently, allowing dissection of the signalling pathways through the collagen binding integrins $\alpha_1(I)$ and $\alpha_2(I)$. The integrin binding protein Uk1, and its role in regulating collagen synthesis was described. Finally, some differences between keloid and scleroderma, and normal fibroblasts were shown, two unknown genes in the pathological cells were shown to be upregulated by tension.

Professor Hideaki Nagase asked how do collagenases interact with the collagen triple helix. Twenty matrix metalloproteinases (MMPs) have been described in humans, the basic structure and function of the MMP family was described. The cleavage site within the triple helix was shown, and experimental mutations of this site in a mouse showed that cleavage could be prevented. Keratinocytes were shown unable to migrate on collagenase resistant collagen. The C-terminal domain of the enzymes was shown to be vital for collagenolysis. The active site of collagenase is too small to hold the native collagen molecule, therefore evidence was provided to show that collagenase is able to unwind collagen to allow cleavage.

Professor Bruce Caterson summarised the Pond-Nuki dog model of osteoarthritis and suggested that there is no therapeutic intervention to slow down cartilage destruction. Numerous experiments were described, showing that proteoglycan release from cartilage occurs at an early stage in *in-vitro* models and is not prevented by MMP inhibitors. These breakdown products of proteoglycan were shown to be cleavage products of aggrecan. Aggrecanase was therefore implied in early cartilage degeneration, with collagen loss and MMP activity occurring subsequent to this. Aggrecanase activity was also discussed in tendon, where it can be stimulated but is actually constitutively expressed. Finally, the role of fatty acids in regulating mediators of cartilage destruction was discussed.

Dr. Jean-Claude Monboisse summarised the characteristics of tumour invasion and suggested that ECM molecules may regulate tumour invasion. Elastin was shown to increase the invasive properties of fibrosarcoma HT1080 cells, while elastin peptides stimulated MMP2 synthesis in these cells. The roles of several other matrix molecules on cell adhesion and proliferation, including laminin-5 and types IV and XVIII collagen were also discussed. In particular, a specific sequence in the $\alpha_3(IV)$ chain has significant effects on tumour cells. The receptors that bind this sequence, CD47

and $\alpha_V\beta_3$, and the regulatory pathways that these receptors mediate were also discussed.

Dr. Michael Maragoudakis suggested an association between hypercoagulability and cancer. Discussed how thrombin may contribute to tumour progression and described several experiments to examine the effects of thrombin on angiogenesis. Showed that endothelial cells stimulated with thrombin are sensitive to VEGF and bFGF, and increase their proliferation and migration. Thrombin was also shown to increase functional KDR receptor and flt-1 mRNA in HUVECs, along with the $\alpha_V\beta_3$ integrin which is involved in tumour progression.

Dr. Ernst Hunziker discussed the role of the extracellular matrix in terms of the biomechanics of a tissue, and described how the physical forces of a tissue can be determined by application of forces to it. Force alters cell metabolism within tissues, and these alterations are specific to the types of forces applied. The effect of compressive force on various intracellular components e.g. the Golgi apparatus and rough endoplasmic reticulum, in cartilage was discussed, along with the effects of high strain rates and high peak stress. A number of experiments of the effects of force on calf cartilage explants were then described.

Dr. Anthony Ratcliffe initially discussed the two classes of implants grown *in-vitro*, one to function immediately, the other to induce tissue repair. The use of vascular grafts was summarised, and the limitations of blood vessel substitutes discussed. The main theme was the development of tissue engineered vascular grafts. The methods by which these were developed and produced was discussed in detail, along with the methods by which they were tested to determine their architecture and mechanical strengths. The result of implantation of these grafts into dogs was shown. Finally, an *in-vitro* tissue called Dermagraft₂ was described, which has been shown to enhance skin wounds and is currently being tested on cardiac tissue that has suffered ischemic damage.

BSMB Meeting
Cellular and Molecular Mechanisms in Tissue Engineering
Hulme Hall, University of Manchester
2/3 April 2001

Monday 2nd April 2001

Registration from 11.00am

12.30 – 14:00 LUNCH

14:00 - 14:40 Tony Ratcliffe, (Advanced Tissue Sciences, La Jolla, USA)
Vascular Graft Development

14:40 - 15:20 Richard Black, (Clinical Engineering, University of Liverpool)
Haemodynamic Modelling in Vitro

15:20 - 16:00 TEA and Posters

16:00 - 16:40 Robert Brown (University College, London).
Fibrin Supports for Tissue Engineering Constructs

16:40 - 17:15 2x15min Short Communications

17:15 BSMB - Annual General Meeting

17:30 Posters

18:30 Buses to Manchester United stadium, Old Trafford for
Conference Dinner

Tuesday 3rd April 2001

- 9:00 - 9:40** **Robert Hawkins**, (Paterson Institute, University of Manchester)
Gene Transfer and Gene Therapy
- 9:40 - 10:20** **Sharon O’Kane**, (School of Biol. Sciences, University of
Manchester)
Dermal wound healing and Stem cells
- 10:20** **COFFEE and Posters**
- 10:50 - 11:20** **Chris Murphy**, (Tissue Engineering Centre, Imperial College,
London) **Chondrogenic differentiation of mesenchymal and
embryonic stem cells**
- 11:20 - 12:20** **4x15min Short Communications**
- 12:20 - 13:40** **LUNCH**
- 13:40 - 14:40** **Brian Ashton** (Robert Jones & Agnes Hunt Orthopaedic
Hospital, Oswestry) **Autologous Chondrocyte Implantation
–Lessons for Tissue Engineering**
- 14:40 - 15:00** **Brian Johnstone**, (Case Western Reserve University, Cleveland,
USA) **Meniscal Cartilage Repair**
- 15:00 - 15:40** **Tony Freemont**, (School of Medicine, University of Manchester)
Strategies for Intervertebral disc repair.
- 15:40** **TEA and depart**

Registration form for the BSMB Spring Meeting**'CELLULAR AND MOLECULAR MECHANISMS IN TISSUE ENGINEERING'**

Organisers: Cay Kielty and Tim Hardingham
Conference venue: Hulme Hall, Oxford Place, Victoria Park, University of Manchester
Conference dinner: At Manchester United Stadium, Old Trafford

Applicant's name: BSMB Member? YES/NO
 Male..... Female.....

Address:

.....

.....

Telephone: Fax: Email:

Bed and breakfast accommodation, and meal requirements:

| | <u>1st April</u> | <u>2nd April</u> | <u>3rd April</u> | <u>Cost (£)</u> |
|---|-----------------------------|-----------------------------|-----------------------------|-------------------------|
| <u>Standard room:</u> £21.50 per night | | | | |
| <u>En-suite room:</u> £32.00 per night | | | | |
| <u>Lunch:</u> £ 7.00 | — | | | |
| <u>Conference dinner:</u> £20.00 (excl. wine) | — | | — | |
| <u>Registration fee:</u> members £15.00 | | | | |
| (incl tea/coffee) nonmembers £25.00 | | | | |
| | | | | <u>Total cost</u> |

DIETARY REQUIREMENTS - Vegetarian YES/NO (circle as appropriate)

Other (please give details)

Please make cheques payable to "BRITISH SOCIETY FOR MATRIX BIOLOGY" and return Registration forms and Abstracts by 28th February 2001to:

Professor Cay Kielty, Telephone: 0161 275 5739
 2.205 Stopford Bulding, Fax: 0161 275 5752
 Oxford Road, Email: cay kielty@man.ac.uk
 Manchester M13 9PT.

TRAVEL INFORMATION

Trains: To Piccadilly or (preferably) Oxford Road Station, Manchester. Frequent buses on Oxford Rd or taxi. See web site map: <http://www.man.ac.uk/welcome/maps.html>

Road: Limited parking places are available, please contact for details. Directions see the web site above.

Instructions for Submission of Abstracts

Abstracts should be submitted on an A4 sheet of paper and structured according to the format below using a 12-point font.

Title in bold

Authors

Affiliations

Introduction

Materials and Methods

Results

Discussion

References

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

The deadline for submission of abstracts is February 28th 2001. Abstracts (4 hard copies PLUS electronic copy, either on floppy disc or by email as an attached file) should be submitted to:

Professor Cay Kielty,
2.205 Stopford Bulding,
Oxford Road,
Manchester M13 9PT.

Telephone: 0161 275 5739

Fax: 0161 275 5752

Email: cay.kielty@man.ac.uk

When you submit your abstract, please indicate the name of Corresponding Author, with Fax, phone and email details.

Also indicate whether you wish your abstract NOT TO BE

- **considered for an ORAL presentation**
- **published in the International Journal of Experimental Pathology (IJEP)**
- **entered into the IJEP poster competition.**

Those selected for oral presentations (10 min + 5 min discussion) will be notified in the first week in March. **Poster boards (1.5m x 1m) and fixings will be supplied.**

MEMBERSHIP SUBSCRIPTION FOR 2001 ARE NOW DUE - PLEASE COMPLETE AUDIT OF MEMBERSHIP DETAILS

Please fill out the following form, EVEN IF NONE OF YOUR DETAILS HAVE CHANGED and return by February 28th 2001 to Dr. Rose Maciewicz, Respiratory and Inflammation Research Department, AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK

In order for the Society to keep accurate records of our membership and those who pay their subscriptions it is necessary for us to ask you to complete these details however they will not be passed onto any 3rd party unless you agree to this.

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