wellcome centre cell-matrix research

MANCHESTER 1824
The University of Manchester

BSMB
BRITISH SOCIETY FOR MATRIX BIOLOGY

Matrix Biology Europe 2018

Celebrating 50 years of FECTS

Manchester, United Kingdom. 21-24 July 2018

INTERNATIONAL SOCIETY FOR MATRIX BIOLOGY ISMB

Kindly sponsored by the ISMB and ASMB

ASMB

Contents

Notes

We thank our sponsors for their kind support for this event.

Welcome Message from the Organisers

Dear MBE2018 Delegates,

On behalf of the British Society for Matrix Biology and The Wellcome Centre for Cell-Matrix Biology, University of Manchester we are delighted to welcome you to the MBE 2018 meeting in Manchester. It is now exactly 50 years since the Federation of European Connective Tissue Societies was formed and the first meeting held in Cambridge, UK. An excerpt from an article by John Scott describing the formation of FECTS and the first meeting is included in your conference guide book. Also included are recollections from the FECTS meeting marking the 20th Anniversary by another founding member of the Federation, Ladislas Robert who has also sadly recently passed away. A principle of FECTS meetings right from the start was the opportunity for younger researchers to present and discuss their work. I hope you agree that we have been able to continue this tradition with many of the short talks and half of the 170 plus posters being presented by early career researchers. We are also delighted to host the Dick Heinegård Young Investigator Award competition where presenters representing 5 different European Matrix Biology Societies will battle it out for the prize in the Tuesday morning session.

We could not have organised this conference without the generous support of a host of sponsors listed in your programme. Please make every effort to visit the different sponsors' stands. In addition, we are grateful for ISMB support and are delighted that ASMB have sponsored one of the workshops at this celebratory meeting. We are also grateful to Marketing Manchester for providing the funding for the Welcome Reception in The Manchester Museum following the opening Plenary on Saturday evening.

We thank the large number of people who have helped with the organisation of this meeting and their names and roles are listed in the programme.

Please participate fully in the meeting and we hope you have a great time.

Ray Boot-Handford & Qing-Jun Meng, local organising committee

John Couchman Chairman BSMB

Excerpt from the account of the first FECTS meeting in 1968. Calc. Tiss. Res. 3, 198--210 (1969)

Federation of European Connective Tissue Clubs

Guest Editorial Connective Tissue Research and Organisation

J. E. SCOTT MRC Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Berks., England

Interest in biological problems in which the properties of macromolecules are of prime importance has tended to focus on the exciting molecular biology of the nucleic acids and certain proteins. Somewhat out of the limelight are those scientists who work on the slimes, jellies, fibres and solids which can be coaxed out of connective tissues. This is a pity, since all the signs point to a very rich, almost untapped, vein of intellectually stimulating problems, which await the attention of biophysicists and others. During evolution from single- celled organisms, remarkable fibre-reinforced composite materials have developed, in which a variety of cells, metabolites and metabolic processes can live, move and have their being. The point was theatrically expressed in a book review, "If by some magic solution, one could at once dissolve all the connective tissue of the body, all that would remain would be a mass of slimy epithelium, quivering muscle and frustrated nerve cells." The drab term 'connective tissue' is a positive deterrent to the bright but uninformed, who might otherwise be strongly attracted to the subject.

Available resources do not match the problems, partly because of the necessity to compete with the demands of other subjects, for instance those in which Nobel prizes are more frequently awarded, or which might produce a break- through in defence strategy. Although many industries (glue, leather, gelatin, cosmetics, etc.) have an interest in connective tissue and its products, relatively little research is done by them, particularly in Europe. The financial cake is too small, as all scientists are aware, and in the slicing up thereof, there is little for the man with pie-in-the-sky. Meanwhile, academic studies are carried out by individuals, or by small groups, often associated with, or peripheral to, other units and organisations engaged on specific problems in rheumatology, dentistry, dermatology, pathology, etc. This dispersion of forces and resources has meant that few people have been aware of how many researchers had full-time interests in this field. The need for communication channels catering for them was not recognised, which made it even more difficult to keep track of what was being done. Paradoxically, in this situation, one had to be well-known to be able to attend the few meetings which were devoted entirely to the subject, since most of them were in the U.S.A., and travel is expensive. It was possible for pre-, and post-, doctoral workers to spend years on their problem without ever seeing an acknowledged leader in their field. This uneconomic, disorganised and sterile situation has begun to improve in the past few years with the formation in various countries of 'clubs', more or less informal, designed to provide maximum contact between 'workers-at-the-bench'. It was a considerable surprise to find just how many wished to participate. At a conservative estimate, not less than three hundred scientists are involved in three British societies. The formation of the Federation of European Connective Tissue Clubs may help to improve the status of workers in this hitherto rather poorly-endowed subject, as well as providing a much-needed forum for discussion and communication. It is time for students of the intercellular matrix to begin to think about the framework in which they work and relate to each other. It would be very surprising if the present ad hoc arrangements could not be improved.

Federation of European Connective Tissue Clubs Proposed

The first meeting of the proposed Federation was held in Cambridge, June 30th to July 2nd 1968. The host clubs were the Collagen Club, and the Mucopolysaccharide Club, of Great Britain, in association with the Bone and Tooth Society. The treasurer was Dr. J. REYNOLDS, the secretary Dr. J. SCOTT, and local organisation in Cambridge was handled by Dr. J. T. DINGLE and Dr. J. REYNOLDS. The meeting was made possible by generous financial help from the Wellcome Foundation, Ethicon Ltd., and J. R. Geigy Ltd.

Sunday, 30th June. Business Meeting. Chairman, Dame JANET VAUGHAN. The suggested aims of the Federation were outlined by Dr. J. REYNOLDS, Dame JANET VAUGHAN and Dr. J. SCOTT. They were: 1) To have a meeting every one or two years which would be self-supporting financially. 2) The clubs participating should retain their identity. 3) No formal organisation or permanent officers were envisaged. …… all organisation of the meeting was to be in the hands of the clubs of the country in which the meeting was to be held. A representative from the country in which the previous meeting was held would serve, along with the organisers of the new meeting, to maintain continuity. 4) No publications were envisaged, except for accounts of the meetings which could appear in already existing journals.

A vote on these proposals, with each person voting individually was carried *nemo*. *con*.

Recollections of the late Professor Ladislas Robert at the 20th Anniversary meeting of FECTS

The French Society for Matrix Biology is profoundly sad for the tragic loss this year of its prestigious founder Professor Ladislas ROBERT. Some of you knew him and appreciated his numerous qualities so human as scientific. Please find below an excerpt from the commemorative speech Ladislas Robert gave for the 20th anniversary of the FECTS meeting in 1988 in Amsterdam. You will probably notice how actual the speech appears despite its date of delivery.

Excerpt from: Tissu Conjonctif (FECTS) – Discours de Mr Ladislas ROBERT au congrès FECTS d'Amsterdam en 1988, publié dans le Bulletin de la Société Française du Tissu Conjonctif (Volume 8, n°4, Octobre 1989), bulletin édité sous la responsabilité de François-Xavier Maquart.

Dear Colleagues,
Let me first thank the organizers, Profs Gruys and Van Den Hoof and their colleagues of the organizing committee Profs Kuyer, Jansen, Van Den Berg, Von Urk and Pierik, for the honor they bestowed upon me by asking me to deliver this talk at the occasion of the celebration of the 20th anniversary of the Federation of European Connective Tissue Societies. As a matter of fact, the spark that started this Federation sprung during a joint meeting we (the French Connective Tissue Club) held with the British Mucopolysaccharide Club whose secretary, John Scott, joined me with all his enthusiasm to make this initiative a two men show. During that meeting, in the Pasteur Institute, in September 1967, we decided to organize the 1st European meeting next year. This event helped me to escape for a few weeks I spent in Cambridge some of the turmoil of the 1968 French student revolution. From 1967 up to the Prague meeting in 1980 where the bylaws of our Federation were approved, we travelled John and I from laboratory to laboratory, country to country to help to make this European Federation a reality. This was not really so difficult because most European Colleagues rapidly joined the movement which also helped to stimulate the Foundation of the National Connective Tissue Societies in more European countries.
It was essential to keep the original format as close as possible to what we and most colleagues believed to be the ideal one, that is an open meeting giving plenty of opportunities to the young scientists to meet each other and the seniors of their field, show their results and practice an uninhibited exchange of thoughts far from the traditional formalism of old fashioned European University practices. The requirement is based on several simple postulates which all are written in the bylaws : a large part of the meeting is reserved for poster presentations, a few formal lectures to bring up-to-date colleagues in vicinal disciplines of the ever larger field of Connective Tissue Research and, very important, to keep it as cheap as possible to enable as many young colleagues, also from eastern countries, to attend. Finally, a business meeting is to be held at every meeting to discuss the site of the next one and propose criticisms and suggestions for improving our format. We also felt that European colleagues had to be served first, but did never close the gate for our overseas colleagues. Since the rapid enlargement of the audience of the FECTS Meetings this is no more felt as a problem, on the contrary, it appears now more and more important to activate the exchange of information among all scientists, European or not.

Committees and Local Organisers

International Advisory Committee

Chairman, John Couchman
(Copenhagen, Denmark)
Nikos Karamanos (Patras, Greece)
Qing-Jun Meng (Manchester, UK)
Taina Pihlajaniemi (Oulu, Finland)
Alberto Passi (Varese, Italy)
Patricia Rousselle (Lyon, France)
Liliana Schaefer (Frankfurt, Germany)
Dimitrios Zeugolis (Galway, Ireland)

Abstract Selection Committee

George Bou-Gharios (Liverpool, UK)
Danny Chan (Hong Kong, China)
Jo Adams (Bristol, UK)
Sylvie Richad-Blum (Lyon, France)
Rachel Lennon (Manchester, UK)
Qing-Jun Meng (Manchester, UK)
John Couchman (Copenhagen, Denmark)
Janine Erler (Copenhagen, Denmark)
James Whiteford (QMUL, UK)
Tim Hardingham (Manchester, UK)
Ray Boot-Handford (Manchester, UK)
Tom Van Agtmael (Glasgow, UK)
Kim Midwood (Oxford, UK)
Dave Thornton (Manchester, UK)
Graham Riley (UEA, UK)
Joe Swift (Manchester, UK)

Local Organizing Committee

Ray Boot-Handford (Manchester, UK)
Qing-Jun Meng (Manchester, UK)

Student organizers:
Aljona Kolmogorova (Manchester, UK)
Nikki-Maria Koudis (Manchester, UK)
Mark Naven (Manchester, UK)
Honor Morris (Manchester, UK)

Fund-raising Committee

Qing-Jun Meng (Manchester, UK)
Ray Boot-Handford (Manchester, UK)
Karl Kadler (Manchester, UK)
Christoph Ballestrem (Manchester, UK)
Peter March (Manchester, UK)
John Couchman (Copenhagen, Denmark)
Liliana Schaefer (Frankfurt, Germany)

Programme

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<tr><td rowspan="11">Registration:
16:00-20:00

Opening Ceremony:
18:00-18:10
Th B

Keynote Lecture:

Reinhard Fässler

18:10-18:50
Th B

Welcome Reception:
19:00-21:00

Manchester Museum</td><td colspan="2">9.00 – 10.00 Plenary 1 Th B

9:00-9:30 Farshid Guilak

9:30-10:00, Janine Erler</td><td colspan="2">9.00 – 9.30 Plenary 3 Th B

9:00-9:30, Joanne Murphy-Ullrich

9:30-10:00, Andy Blanchard</td><td>9:00-10:45, Dick Heinegård Young Investigator Award presentations (5x15') Th B</td></tr>
<tr><td colspan="2">10:00-10:45 Coffee break</td><td colspan="2">10:00-10:45 Coffee break</td><td>10:45-11:30 Coffee break</td></tr>
<tr><td>10:45-12:30

Workshop 1 Th B

Stem cells and Matrix Engineering</td><td>10:45-12:30

Workshop 2 Th A

Fibrillar/Matri-cellular Signalling</td><td>10:45-12:30

Workshop 5 Th A

ASMB sponsored session: Pathobiology & Therapeutics to</td><td>10:45-12:30

Workshop 6 Th B

Mechanisms of Matrix Disease</td><td>11:30-12:30 Th B

PL5 (Erhard Hohenester)

2x15' Hot topic STs</td></tr>
<tr><td colspan="2">12:30-14:30 Lunch/Posters

(14:15-14:30 Group photo)

(13:00 -14:00 ISMB Council Meeting, Rm 3.205 Uni Place)</td><td colspan="2">12:30-14:30 Lunch/Posters

(14:00-14:30 BSMB AGM)</td><td>12:15-13:30 Th B
MBE AGM/ Prizes/Closing ceremony/Lunch</td></tr>
<tr><td>14:30-16:10

Workshop 3 Th A

Rhythms and Matrix Dynamics</td><td>14:30-16:10

Workshop 4 Th B

ECM Micro-environment, Adhesion and Cell Fate</td><td>14:30-16:10

Workshop 7 Th A

The Immunology/

Matrix Interface</td><td>14:30-16:10

Workshop 8 Th B
Matrix Mechano-biology</td><td rowspan="5">14:30-18:00

BSMB Committee meeting

(2.57 Simon Building)

(STs, selected talks)</td></tr>
<tr><td colspan="2">16:10-16:40 Tea break</td><td colspan="2">16:10-16:40 Tea break</td></tr>
<tr><td colspan="2">Plenary 2 16.40 – 18.00 Th B

16:40-17:10 Taina Pihlajaniemi</td><td colspan="2">16:40-17:30 Plenary 4 Th B

BSMB Fell Muir Award with reception afterwards</td></tr>
<tr><td colspan="2">17:10-18:00 ISMB Session including Rupert Timpl Award with reception afterwards</td><td colspan="2">17:30-18:00 (MBE Reps committee meeting, Rm 5.205 in University Place)</td></tr>
<tr><td colspan="2">Free time

Post-doc/PhD social</td><td colspan="2">19:30-23:00

Conference Dinner (Old Trafford)</td></tr>
</table>

MBE2018 Manchester – Celebrating 50 years of FECTS meetings
Final Programme

Saturday 21st July 2018

16.00-20.00 Registration (University Place, Oxford Road)

Opening Session & Keynote Lecture (Theatre B)

Chairperson: Ray Boot-Handford

18.00 – 18.10 Opening Remarks and Welcome

18.10 – 18.50 Reinhard Fässler (MaxPlanck, Martinsried, Germany) Keynote Address, 'The Kindlins and their functions'

19.00 – 21.00 **Welcome Reception in Manchester Museum with a welcome address from The Lord Mayor of the City of Manchester, Councillor June Hitchen.**

Sunday 22nd July 2018

Plenary Session 1 (Theatre B)

Chairperson: Kim Midwood

9.00 – 9.30 Farshid Guilak (Washington Uni, USA): 'Engineering new biologic therapies for arthritis'

9.30 – 10.00 Janine Erler (BRIC Copenhagen, Denmark): 'ECM remodelling during cancer progression'

10.00 – 10.50 Coffee break

10.50 – 12.30 Workshop 1: Stem cells and Matrix Engineering (Theatre B)

Chairperson: John Bateman

10.50 – 11.10 Gerjo van Osch (Erasmus MC, Netherland): 'Adult human Mesenchymal Stem Cells; heterogeneity and cartilage matrix engineering capacity'

11.10 – 11.25 (**P9**) Tom Hodgkinson (U of Glasgow, UK) Identification and in vitro screening of osteogenic metabolites through supplement-free nanovibration-driven mesenchymal stem cell differentiation

11.25 – 11.40 (**P8**) Wai Kit Tam (HKU, Hong kong) Deriving Nucleus Pulposus-like Progenitors from MAP kinase Interference Coupled Chondrogenic Induction in Mesenchymal stem cells

11.40 - 11.55 (**P7**) Stefanie Korntner (NUI Galway, Ireland) In vivo wound healing with an engineered matrix-rich living modular construct for stem cell delivery
11.55 – 12.10 (**P4**) Aggie Turlo (U of Liverpool, UK) Insulin-like growth factor binding protein (Igfbp6) is a cross-species transcriptomic tendon marker
12.10 – 12.30 Dimitrios Zeugolis (NUI Galway, Ireland): 'Extracellular matrix rich supramolecular assemblies in regenerative medicine'

10.50 - 12.30 Workshop 2: Fibrillar/Matricellular Signalling (Theatre A)
Chairperson: Patricia Rousselle
10.50 – 11.10 Laurent Duca (Reims, France): 'Elastin modification during vascular aging and pathophysiological consequences'
11.10 – 11.25 (**P20**) Michael P. Lockhart-Cairns (WCCMR, Manchester UK) The Structure and Regulation of latent TGFβ by LTBP1 and Fibrillin
11.25 – 11.40 (**P25**) Sylvie Ricard-Blum (U of Lyon.1, France) Insights into the structure and dynamics of lysyl oxidase propeptide, a flexible protein with numerous partners
11.40 - 11.55 (**P27**) Suneel Apte (Cleveland, USA) Recycled secreted metalloproteases are required for formation of the primary cilium and hedgehog signalling
11.55 - 12.10 (**P19**) Giulia Taraboletti (IRCCS, Bergamo Italy) The interaction of thrombospondin-1 and -2 with FGF2 in the control of tumor angiogenesis
12.10 – 12.30 Wei Kong (PKUHSC, Beijing China): 'Cartilage Oligomeric Matrix Protein Interactome in Vascular Homeostasis'

12.30 – 14.30 LUNCH and POSTERS (15 min lunch time workshop from key sponsors: Kristian Wadel (FEI Electron Optics) 'Large Volumetric Analysis of Tissue Ultrastructure')

14.30 – 16.10 Workshop 3: Rhythms and Matrix Dynamics (Theatre A)
Chairperson: Qing-Jun Meng
14.30 – 14.50 Karl Kadler (WCCMR, Manchester UK) 'On the existence of a circadian matrix that is mechanoprotective and able to respond quickly to injury'
14.50 – 15.05 (**P44**) Yutaka Matsubayashi (Kings, London, UK) Distribution and production logistics for de novo basement membrane formation

15.05 – 15.20 (**P45**) Michal Dudek (WCCMR, Manchester UK) The role of the circadian clock in the homeostasis of the extracellular matrix in cartilage and intervertebral discs
15.20 – 15.35 (**P37**) Vanja Pekovic-Vaughan (U of Liverpool, UK) Abnormal fibrillinogenesis leads to disruption of clock gene regulation and reveals an important role for fibrillar matrix in the maintenance of circadian rhythmicity
15.35 – 15.50 (**P43**) Adam Pickard (WCCMR, Manchester UK) Monitoring collagen fibre formation and turnover using CRISPR/Cas9 knock-in of Dendra2
15.50 – 16.10 Kazuhiro Yagita (Kyoto, Japan): 'Regulation and mis-regulation of cellular differentiation-coupled circadian clock functionality in mammals'

14.30 – 16.10 Workshop 4: ECM Microenvironment, Adhesion and Cell Fate (Theatre B)
Chairperson: James Whiteford & Laura Collins
14.30 – 14.50 Christa Maes (KU Leuven, Belgium): 'Osteolineage Cells'
14.50 – 15.05 (**P73**) Hong Qian (Karolinska, Stockholm Sweden) Laminin α4 deletion leads to impaired hematopoietic regeneration following irradiation-induced injury and accelerates the progression of acute myeloid leukemia
15.05 – 15.20 (**P67**) Adam Byron (U of Edinburgh, UK) Kindlin-1 regulates the mammary tumour cell secretome
15.20 – 15.35 (**P71**) Kati Drushinin (Biocentre Oulu, Finland) Collagen prolyl 4-hydroxylases in collagen fibril formation and wound healing in mouse skin
15.35 – 15.50 (**P65**) Satsuki Mochizuki (Juntendo Univ, Japan) Development of human antibody against ADAM28, a key modulator of tumor microenvironmental factors in non-small cell lung carcinomas
15.50 – 16.10 Alberto Passi (U of Insubria, Italy): 'Epigenetic control of hyaluronan synthesis'

16.10 – 16.40 Tea Break

Plenary Session 2 (Theatre B)
Chairperson: Liliana Schaefer
16.40 – 17.10 Taina Pihlajaniemi (U of Oulu, Finland): Collagen XVIII and its contributions to development of tissues and tumourigenesis
17:10 – 17:20 ISMB Award to David Hulmes

17.20 – 18.00 ISMB Rupert Timpl Award Lecture, Sponsored by Elsevier / Matrix Biology (followed by Reception)

Alexandra Naba (Uni of Illinois at Chicago, USA) The Matrisome Project: bioinformatic and proteomic tools to study the ECM in health and disease

Monday 23rd July

Plenary Session 3 (Theatre B)

Chairperson: Sylvie Ricard-Blum

9.00 – 9.30 Joanne Murphy-Ullrich (U of Alabama at Birmingham, USA): The ER stress and calcium regulatory protein calreticulin in fibrosis in diabetic nephropathy

9.30 – 10.00 Andy Blanchard (GSK Stevenage, UK): 'Translating research to therapies for lung fibrosis'

10.00 – 10.50 Coffee break

10.50 – 12.30 Workshop 5: ASMB sponsored session: Pathobiology and Therapeutics to Fibrosis (Theatre A)

Chairperson: Joanne Murphy-Ullrich

10.50 – 11.10 Rebecca Wells (UPenn, USA): 'The effect of a complex matrix on the mechanics of liver fibrosis'

11.10 – 11.25 (**P103**) George Bou-Gharios (U of Liverpool, UK) Ubiquitous removal of ccn2 in a bleomycin induced pulmonary fibrosis model is detrimental to survival

11.25 – 11.40 (**P107**) Valentina Masola (U of Padova, Italy) Inhibition of heparanase protects against renal failure and fibrosis following ischemia/reperfusion

11.40 - 11.55 (**P110**) Marc Tatar (Brown Univ, USA) Extra-cellular matrix induced by aldosterone through a G-protein coupled receptor revealed in a novel Drosophila model of renal fibrosis

11.55 - 12.10 (**P102**) Shaun Fell (Aston Univ, UK) The importance of tissue transglutaminase for the deposition of matrix proteins in idiopathic pulmonary fibrosis

12.10 – 12.30 Tom Barker (U of Virginia, USA): 'Post-translational modifications of Fibronectin as therapeutic targets for Fibrosis?'

10.50 – 12.30 Workshop 6: Mechanisms of Matrix Disease (Theatre B)

Chairperson: Kathy Cheah

10.50 – 11.10 Nikos Karamanos (U of Patras, Greece): 'ERs as regulators of ECM: from epigenetics to breast cancer cell behaviour'

11.10 – 11.25 (**P126**) Francesca Tonelli (U of Pavia, Italy) New Zebrafish models for recessive osteogenesis imperfecta

11.25 – 11.40 (**P122**) Jamie Soul (WCCMR, Manchester UK) Understanding skeletal disease with cross-species transcriptomics meta-analysis

11.40 - 11.55 (**P139**) Anna Köhler (U of Cologne, Germany) Ablation of epidermal collagen chaperoning by Hsp47 results in dermal fibrosis

11.55 - 12.10 (**P127**) Johanne Dubail (INSERM UMR1163, Paris France) SLC10A7 mutations in human and mouse cause a skeletal dysplasia with amelogenesis imperfecta mediated by GAG biosynthesis defects.

12.10 – 12.30 Mike Briggs (U of Newcastle): 'New therapeutic targets in genetic skeletal diseases'

12.30 – 14.30 LUNCH and POSTERS

14.30 – 16.10 Workshop 7: The Immunology/Matrix Interface (Theatre A)

Chairperson: John Whitelock

14.30 – 14.50 Liliana Schaefer (Goethe Univ, Germany): 'Small leucine-rich proteoglycans in inflammation: two sides of the coin'

14.50 – 15.05 (**P156**) Victor Martinez (UCL, UK) Leukocyte control of matrix deposition in the lymph node

15.05 – 15.20 (**P158**) Anja Schwenzer (KIR, Oxford Univ, UK) Post-translational modifications of the extracellular matrix: key events in disease pathogenesis

15.20 – 15.35 (**P154**) Caroline Milner (U of Manchester, UK) TSG-6 modulates chondrocyte phenotype in osteoarthritis by suppressing inflammatory signals that promote cartilage breakdown

15.35 – 15.50 (**P155**) Douglas Dyer (U of Glasgow, UK) Leukocyte migration and inflammatory disease: the collaboration between endothelial sugars and chemokines

15.50 – 16.10 Judi Allen (WCCMR, Manchester UK): 'Regulation of Matrix by Type 2 cytokines: Learning from Helminths'

14.30 – 16.10 Workshop 8: Matrix Mechanobiology (Theatre B)

Chairperson: Checco Ramirez & Michal Dudek

14.30 – 14.50 Viola Vogel (U of Zürich, Switzerland) "Mechanobiology of Extracellular Matrix Fibers in vitro and in vivo"

14.50 – 15.05 (**P171**) Mukti Singh (WCCMR, Manchester UK) Structure and interactions of elastic fibre proteins ADAMTSL2 and ADAMTSL4.

15.05 – 15.20 (**P165**) Emma Blain (Cardiff Univ. UK) Mechano-regulation of miRNA-221, -222, -21 and -27: implications for articular cartilage homeostasis

15.20 – 15.35 (**P167**) Marion Marchand (CNRS Paris, France) Extracellular matrix scaffolding: impact on vascular morphogenesis and endothelial mechanotransduction

15.35 – 15.50 (**P172**) Joe Swift (WCCMR, Manchester UK) Nuclear decoupling is part of a rapid protein-level cellular response to high-intensity mechanical loading

15.50 – 16.10 Maria Dolores Martin Bermudo (Sevilla, Spain): 'Cellular contractility controlled by cell-ECM interactions sculpt organs and tissues'

16.10 – 16.40 Tea Break

16.40 – 17.30 Plenary Session 4 (Theatre B)

BSMB Fell-Muir Award, sponsored by The Int. Journal Experimental Pathology (followed by Reception)

Chairperson John Couchman

Ray Boot-Handford (WCCMR, Manchester UK): Gene cloning to clinical trials - the trials and tribulations of a life with collagen

18.30 Manchester United Stadium Tour

19.30 Conference Dinner at MU Stadium

Details of coaches to and from stadium will be given at the conference.

Tuesday 24th July 2018

9.00 – 10.45 Dick Heinegård Young Investigator Award Session (Theatre B)

9.00 – 9.05 Introductory comments by Chairman of Heinegård YIA committee

9.05 – 9.25 **Raphael Reuten** representing **The German Society for Matrix Biology** BRIC, University of Copenhagen, Denmark

"Modulating the physico-mechanical properties of basement membranes through the extracellular-matrix protein netrin-4"

9.25 – 9.45 **René Brüggebusch Svensson** representing **The Danish Society for Matrix Biology** Institute of Sports Medicine Copenhagen, Denmark

"Mechanical properties of collagen fibrils in tendon"

9.45 – 10.05 **Andrea Lolli** representing **The Dutch Society for Matrix Biology** Erasmus University Medical Center, Netherlands

"Hydrogel-based delivery of antiMir-221 enhances cartilage matrix production by endogenous cells: towards microRNA therapy for cartilage repair"

10.05 – 10.25 **Ching-Yan Chloé Yeung** representing **The British Society for Matrix Biology** Institute of Sports Medicine Copenhagen, Denmark

"Regulation of tissue homeostasis by the tendon clock"

10.25 – 10.45 **Karl Emil Tykesson** representing **The Swedish Connective Tissue Society** Lund University, Lund, Sweden

"Crystal structure of dermatan sulfate epimerase 1 and its interaction with dermatan 4-sulfotransferase 1"

10.45 – 11.30 Coffee Break

11.30 – 12.30 Plenary Session 5 (Theatre B)

Chairperson Karl Kadler

11.30 – 12.00 Erhard Hohenester (Imperial College London): 'Biosynthesis and function of (honorary) glycosaminoglycans: insights from structural studies'

Hot Topics

12.00 – 12.15 (**P64**) Frank Gondelaud (U of Lyon 1, France) Structural characterization of the ectodomains of the four human syndecans

12.15 – 12.30 (**P118**) Tom van Agtmael (U of Glasgow, UK) Targeting ER stress in Col4a1 mutant mice reduces intracerebral haemorrhaging and highlights tissue specific disease mechanisms

12.30- 13.10 MBE AGM, Prizes, closing ceremony

Chairman John Couchman BSMB

13.10 – 13.40 Lunch and departure

14:00 – 18:00 Satellite Workshop run by Alvéole, in collaboration with Manchester University (Michael Smith Building) -- Further details here

Speaker Summaries and Abstracts

Keynote Lecture

The Kindlins and their functions

Reinhard Fässler
Max Planck Institute of Biochemistry, Department of Molecular Medicine, Martinsried, Germany

Integrins are α/β heterodimeric receptors that mediate cell-extracellular matrix (ECM) and cell-cell interactions. In order to interact with their ligand, integrins must shift from inactive to active conformations. Talin and Kindlin stabilize the active conformation of integrins by directly binding integrin tails and linking integrins with the actomyosin system. At the meeting I will discuss novel *in vivo* functions of the integrin tail-associated protein Kindlin.

Plenary Session 1

Engineering new biologic therapies for arthritis

Farshid Guilak, Ph.D.
Professor and Director
Center of Regenerative Medicine and Shriners Hospitals for Children – St. Louis
Departments of Orthopaedic Surgery, Developmental Biology, and Biomedical Engineering
Washington University, St. Louis MO

Osteoarthritis is a painful and debilitating disease of the joints that is characterized by progressive degeneration of the articular cartilage; however, there are currently few disease-modifying treatments available. In recent years, the advent of synthetic biology and gene-editing methods such as CRISPR-Cas9 has allowed for precise modifying gene networks that control cell behavior. We have applied a combination of principles from these fields to rewire cellular gene circuits in a manner that allows us to create a unique, custom-designed cell type that can sense and respond to its biochemical environment in a pre-programmed way. These cells have been used to develop engineered tissue replacements with the ability for tunable, inducible, or feedback-controlled, auto-regulated biological responses. In addition to recapitulating the biochemical and biomechanical properties of the tissue, these "smart" constructs can provide controlled drug delivery and immunomodulatory responses to the joint as therapies for osteoarthritis or rheumatoid arthritis.

Targeting cell-ECM interactions to disrupt metastasis

Janine T. Erler
BRIC, University of Copenhagen

Metastasis is strongly regulated by the extracellular matrix (ECM). Studies have shown that the ECM can promote or restrict cancer progression, and that targeting cell-ECM interactions can disrupt tumour growth and invasion as well as improve drug response. Very little is known about the ECM at the metastatic niche. We have taken several approaches to characterise metastatic ECM, study how it regulates cell behaviour, and target cell-ECM interactions to disrupt metastatic tumour formation and growth. We aim to translate our findings into the clinic to improve the survival of patients with metastatic disease.

Workshop 1: Stem cells and Matrix Engineering

Adult human Mesenchymal Stem Cells heterogeneity and cartilage matrix forming capacity

Gerjo J.V.M. van Osch
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Mesenchymal Stem Cells (MSC) are a population of cells that can be obtained from adult tissues and expanded in culture to obtain a large number of cells with multi-lineage differentiation capacity. Thus MSC offer great promise for tissue engineering.
One of the limiting factors of the use of MSC is their heterogeneity. This leads to variable outcomes between patients, labs and experiments. Part of the heterogeneity can be explained by the origin of the MSC. Besides differences caused by harvesting site, different types of MSC are present within one harvest. We have demonstrated differences in chondrogenic capacity between perivascular and lining cells from bone marrow as well as from synovium.
Further heterogeneity is caused by in vitro expansion. Cell expansion is known to cause cellular senescence and loss of differentiation potential. The method of expansion is of large influence and we have found that addition of Wnt3a to the culture medium improves the quality of the MSC.
Future research to better understand the heterogeneity of this population of cells and the potential of its subpopulations, will improve the application of MSC in disease model systems and regenerative medicine application and might help us to find methods to stimulate endogenous tissue repair.

Extracellular matrix rich supramolecular assemblies in regenerative medicine

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Cell therapies aspire to restore lost function by exploiting the inherent capacity of cells to build highly sophisticated supramolecular tissue assemblies. During *in vitro* expansion, bereft of their optimal tissue milieu, cells lose their phenotype, function and therapeutic potential. Although various biophysical *in vitro* microenvironment modulators (e.g. surface topography, substrate rigidity) have been shown to control cell function *in vitro*, they do not increase extracellular matrix (ECM) deposition. Consequently, clinical translation and commercialisation of tissue engineered tissue-like assemblies remains onerous, considering that the development of such implantable device in the highly dilute culture media takes several weeks to months. Macromolecular crowding (MMC), the addition of macromolecules in the culture media, is a biophysical phenomenon that regulates the intra- and extra- cellular environment of multicellular organisms and increases thermodynamic activities and biological processes by several orders of magnitude. Herein, the influence of MMC, alone or in combination with other *in vitro* microenvironment modulators (e.g. physiological for the cells oxygen tension and mechanical stimulation) in permanently differentiated and stem cell cultures for the accelerated development of ECM-rich tissue-like assemblies will be discussed.

Workshop 2: Fibrillar/ Matricellular Signalling

Elastin modification during vascular aging and pathophysiological consequences

Laurent DUCA
UMR CNRS / URCA 7369, "Extracellular Matrix and Cell Dynamics" (MEDyC), Team 2 "Matrix Aging and Vascular Remodelling", Reims, France

Cardiovascular diseases (CVD) are the leading cause of death worldwide and represent a major problem of public health. Over the years, life expectancy has considerably increased throughout the world and the prevalence of CVD is inevitably rising with the growing aging of the population. The normal process of aging is associated with progressive deterioration in structure and function of the vasculature, commonly called vascular aging. At the vascular level, extracellular matrix (ECM) aging leads to molecular alterations of long half-life proteins, such as elastin and collagen, and have critical impacts on vascular diseases. Elastin, is an insoluble and hydrophobic protein mainly produced by smooth muscle cells in the media and fibroblasts in the adventitia. As elastogenesis is restricted to fetal and infancy, mature elastin fibers remain for lifespan. Indeed, its strong reticulation makes elastin a highly stable molecule with longevity comparable with human lifespan and any proteolytic damage that does occur with age and disease is essentially irreparable. Under pathological conditions, vascular and inflammatory cells produces elastases able to degrade elastin, leading to the production of elastin derived peptides (EDP) that actively participate through the Elastin Receptor Complex (ERC) to the development of diseases such as diabetes, atherosclerosis, thrombosis and vascular calcification. Consequently, the proactive role of the ECM suggests that the development of a prognostic value and an innovative therapy based on matrix biology has a strong potential. The works presented here will summarize and discuss these points.

Cartilage Oligomeric Matrix Protein Interactome in Vascular Homeostasis

Wei Kong
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Cartilage oligomeric matrix protein (COMP), also known as thrombospondin-5 (TSP-5), is a matricellular protein that is abundantly expressed in both cartilage and the cardiovascular system. We have identified a series of COMP binding proteins, such as metalloproteinase ADAMTS-7, integrin α7β1, and bone morphogenetic protein 2 in vascular smooth muscle cells, integrin β3 in macrophage, thrombin in platelet et al in the cardiovascular system and therefore playing important roles in maintaining cardiovascular homeostasis. Herein we also found COMP-prohibitin 2 interaction maintains mitochondrial homeostasis and controls smooth muscle cell identity. Additionally, COMP is a novel notch ligand driving embryonic stem cell differentiation towards the smooth muscle lineage.

Workshop 3: Rhythms and Matrix Dynamics

Circadian Clock Regulation of the Secretory Pathway

Ching-Yan Chloé Yeung[1,2]¥, Richa Garva[1]¥, Adam Pickard[1], Joan Chang[1], David F. Holmes[1], Yinhui Lu[1], Venkatesh Mallikarjun[1], Joe Swift[1], Antony Adamson[1], Ben Calverley[1], Oliver Jensen[3], Tom Shearer[3], Qing Jun Meng[1]* and Karl E. Kadler[1]*

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Proteins destined for secretion move from the endoplasmic reticulum (ER, the site of synthesis) to Golgi cisternae then to the cell surface in transport vesicles. How temporal coordination of transport is coordinated with physiological demands of tissues is not understood. Here we show that the levels of intracellular collagen-I (the most abundant secreted protein in vertebrates) are rhythmic over a 24-hour cycle in tendon, the tissue richest in collagen-I and having diurnal extremes of physiological loading. This rhythmicity results from the circadian control of transport between each node in the secretory pathway, commencing with translocation into ER, to transfer to Golgi, and finally release to the extracellular matrix. Thus, the circadian clock is a master logistic operator of the secretory pathway. This is further confirmed by ordinary differential equation modelling using data generated here, which accurately predicted the level of collagen-I protein throughout the day.

Regulation and mis-regulation of cellular differentiation-coupled circadian clock functionality in mammals

Kazuhiro Yagita

Department of Physiology and Systems bioscience, Kyoto Prefectural University of Medicine

Circadian clocks entrain the daily fluctuations of essential biological processes from the molecular to organismal levels to predict and adapt to the cyclic environment of our rotating planet. Cell-autonomous circadian clocks exist throughout the body, which function as an interface connecting cyclic environmental changes to the cellular physiology.

Recently, we found that mouse embryonic stem cells (ESCs) do not display discernible circadian molecular oscillations, whereas circadian molecular oscillation is clearly observed in *in vitro*-differentiated ESCs, indicating that circadian clock development in mammalian cells is closely correlated with the cellular differentiation process.

Discovery of close link with the cellular differentiation has been expanding physiological and pathophysiological implications of circadian clock. In this presentation, I would like to introduce our series of studies investigating from the view point of the correlation between circadian clock and cellular differentiation.

Workshop 4: ECM Microenvironment, Adhesion and Cell Fate

Osteolineage Cells in Bone Development, Homeostasis and Regeneration

Christa Maes
Laboratory of Skeletal Cell Biology and Physiology (SCEBP), Skeletal Biology and Engineering Research Center (SBE), Department of Development and Regeneration, KU Leuven, Leuven, Belgium

Mature, bone-forming osteoblasts represent principle mediators of skeletal development, growth, homeostasis, and repair by being responsible for bone matrix deposition and mineralization. Cells of the osteoblast lineage also contribute to the regulation of hematopoiesis, by constituting essential components of the hematopoietic stem cell niches within the bone and bone marrow environment. In addition, osteoblasts play important roles in the control of whole-body energy metabolism.
To fulfill their key functions, osteoblasts need to differentiate into matrix-producing and mineralizing cells adhering to the bone matrix, from mesenchymal stem or progenitor cells that initially commit to the osteoblast lineage as osteoprogenitors. Accordingly, indispensable aspects of bone formation include the recruitment and engagement of progenitors with osteogenic potential, their migration towards and attachment on the bone surface at sites in need of bone formation, and their proper differentiation and activation into functional osteoblasts. These processes occur in tight temporal and local synchronization with skeletal blood vascularization, referred to as osteo-angiogenic coupling.
A better understanding of the endogenous osteogenic progenitor cells in the bone and bone marrow environment, and of their interplay with the endothelial cells of the bone vascular system, may contribute to the development of new osteo-anabolic therapies for widespread low bone mass disorders such as osteoporosis, and of therapeutic intervention strategies in situations of compromised fracture healing.

Epigenetic control of hyaluronan synthesis

Alberto Passi
University of Insubria, Italy

No summary received

Plenary Session 2

Collagen XVIII and its contributions to development of tissues and tumourigenesis

Taina Pihlajaniemi, Ritva Heljasvaara, Valerio Izzi, Sanna-Maria Karppinen, Raman Devarajan, Inderjeet Kaur, Vanessa Lopez Heikkinen, Tiina Petäistö, Juho Lakkala and Riku Kallunki
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The 28 different collagens (Col) form a major ECM protein family marked by extraordinary functional diversity, ranging from structural support to tissues to critical regulatory binding activities and biologically active cryptic domains releasable through ECM proteolysis. Collagen XVIII (ColXVIII) is a non-fibrillar collagen and proteoglycan which belongs to a small subgroup of basement membrane-associated multiplexins (multiple triple-helix domains with interruptions) within the collagen superfamily. Mammalian ColXVIII is produced as three variant chains, which differ from each other in terms of their N-terminal domain structure and tissue distribution. ColXVIII has important roles in the eye and the brain and the clinical spectrum is expanding. ColXVIII is upregulated in several types of stem cells (SCs), including hair follicle and hematopoietic SCs, and breast cancer SCs, hence suggesting roles in regulating SC properties and function. Studies with mouse models show that ColXVIII is required for proper development of adipose tissues and hair follicles and the establishment of hematopoietic hierarchy. Studies with experimental tumour models and patient samples highlight further the roles of this collagen in tumourigenesis.

ISMB Rupert Timpl Award Lecture

The Matrisome Project:
Bioinformatic and Proteomic Tools to Study the ECM in Health and Disease

Alexandra Naba, Ph.D.
Department of Physiology and Biophysics - University of Illinois at Chicago

Degradation, hyper-production or alteration of the composition of the ECM causes or accompanies numerous pathologies including musculo-skeletal and cardio-vascular diseases, fibroses, and cancers. Thus, a better characterization of ECM composition, metabolism, and biology can lead to the identification of novel biomarkers and therapeutic opportunities. In the first part of my talk, I will describe new developments in bioinformatics and mass-spectrometry-based proteomics to define the *in silico* and *in vivo* “matrisomes” of normal and diseased tissues. I will then present the upcoming release of the second version of the ECM Atlas, a compendium of proteomic data on the ECM of normal and diseased tissues. In the last part of my talk, I will discuss how ECM proteomics can be applied to human cancer patient samples and permit the identification of novel prognostic and diagnostic markers.

Plenary Session 3

The ER stress and calcium regulatory protein calreticulin in fibrosis in diabetic nephropathy

Ailing Lu, Manuel A. Pallero, Benjamin Owusu, Anton Borovjagin, Paul Sanders#, and Joanne E. Murphy-Ullrich
Departments of Pathology and Medicine#, University of Alabama at Birmingham, Birmingham, AL, USA

Endoplasmic reticulum (ER) stress is a contributing factor to fibrotic diseases. Previously, we showed that the ER chaperone and calcium regulatory protein, calreticulin (CRT), is important for collagen transcription, secretion, and assembly into the ECM. Furthermore, CRT is critical for TGF-β stimulation of type I collagen transcription through regulation of ER calcium release and NFAT activation. Using renal-targeted ultrasound delivery of cre-recombinase plasmid to knockdown CRT in kidneys of diabetic *Calr* $^{fl/fl}$ mice, we observed reduced albuminuria and improved survival, and reduced renal fibrosis and collagens I and IV. siRNA CRT knockdown reduced soluble collagen in HK-2 cells stimulated with TGF-β or high glucose. CRT knockdown kidneys had reduced nuclear NFAT in renal tubules and treatment of diabetic mice with 11R-VIVIT, an NFAT inhibitor, reduced proteinuria. These studies identify CRT as an important regulator of ECM production in the diabetic kidney, potentially through regulation of NFAT-dependent ECM transcription.

The Influence of ECM on Heterogeneity in IPF: from Man to Models

Andy Blanchard
GSK Stevenage, UK

IPF is a complex and heterogeneous disease potentially reflecting alternate cell types and mechanisms influencing induction and disease progression. Further diversity exists based on ECM turnover and disease progression rates which are presumably inter-related. Little of this variability in disease endotypes has been used in selecting patients for clinical studies contributing to unusually poor success rates to date.
The expression of neo-epitopes of ECM proteins may provide important clues to not only which patients to target but important mechanisms underlying disease progression. Increasingly the ECM itself is seen as a key driver of chronic disease progression and matrix turnover may generate important matrikines in a disease context. Recent clinical biomarker studies have identified a number of ECM neo-epitopes that differentiate patient endotypes with likely, and in some cases proven, differences in clinical response to therapies. It is exciting to speculate that we may be able to translate some of this important clinical heterogeneity into pre-clinical experiments that can inform importantly on target and asset selection and even clinical dose prediction, given recent advances in the generation of viable precision cut lung slices (PCLS).

Workshop 5: ASMB sponsored session: Pathobiology and Therapeutics to Fibrosis

The effect of a complex matrix on the mechanics of liver fibrosis

Rebecca G. Wells
University of Pennsylvania
Philadelphia, PA USA

The liver, like most organs in the body, is highly cellular but poor in matrix in the normal state, and demonstrates increasing amounts of matrix in the fibrotic state. Detailed mechanical studies show that the relationship between stiffness and collagen content is not linear and that collagen cross-linking is a significant determinant of stiffness. As fibrosis progresses to cirrhosis, there are large-scale architectural rearrangements beginning with the formation of fibrous bridges and progressing to cirrhosis. We report that mechanical factors play a major role in determining the function of the fibrotic liver and drive architectural changes in late fibrosis; additionally, we have found that the liver, like other organs, compression stiffens and that this occurs within a physiologically-relevant range. Findings related to both baseline mechanical properties of the liver and the role of mechanics and matric composition in architectural changes will be presented.

Extracellular matrix memory directs stromal cell signaling and behavior

Thomas H. Barker
University of Virginia

Complex human diseases like cancer and fibrosis do not result from a single acute event or disruption, but rather multiple subcritical hits yet, the extent to which such events are recorded and kept score within a tissue is not obviously apparent. We have now demonstrated that the extracellular matrix protein fibronectin, through at least two different posttranslational modifications, has the capacity to record memories of acute events and influence future cellular behaviors. During inflammatory insults, fibronectin is specifically modified, citrullinated, by neutrophilic and macrophage derived peptidyl arginine deiminases in at least 24 locations. Additionally, fibronectin demonstrates a unique ability to record its own mechanical loading history, again through the actions of a posttranslational modification. These recordings are, to our knowledge, irreversible in the ECM apart from ECM remodeling and exert a significant impact on future stromal cell behaviors by triggering specific integrin switches. These two mechanisms highlight the potential of the ECM to store memories, a surprising phenomenon that will undoubtedly impact our understanding of complex diseases but also the success of stem cell-based therapies and decellularized tissue for regenerative medicine.

Workshop 6: Mechanism of Matrix Disease

ERs as regulators of ECM: from epigenetics to breast cancer cell behaviour

Z Piperigkou[1,2], K Kyriakopolou[1], E Kefali[1], M. Franchi[3], NK Karamanos[1,2]
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Introduction
In order to elucidate the role of estrogen receptors (ERα/β), the implication of EGFR and key matrix effectors in breast cancer development and progression, a new breast cancer cell line has been established; the ERβ-suppressed MDA-MB-231 cells (shERβ MDA-MB-231). MicroRNAs play important role in mammary caner development. Therefore, in this study we evaluated the importance of ERs covering the axis from the epigenetics (microRNAs) to EGFR signalling in the expression of matrix macromolecules and cell behaviour.
Materials and Methods
Control and stably transfected shERβMDA-MB-231 cells used to evaluate to effect of ERβ as well as the importance of EGFR pathway in the presence and absence of E2 and EGFR specific inhibitor in the expression of matrix effectors, cell morphology and EMT. Moreover, the impact of ERs on the microRNAs expression levels in breast cancer cell lines with different ER status (MCF-7 and MDA-MB-231 before and after suppression of ERβ and/or microRNAs transfections have been evaluated.
Results
ERβ suppression induces significant phenotypic changes and alterations in gene and protein expression of major ECM macromolecules (MMPs, syndecans, plasminogen activation system components) and the functional properties of MDA-MB-231 breast cancer cells. Notably, EGFR inhibition in shERβMDA-MB-231 breast cancer cells triggered striking changes in functional properties (migration, proliferation, adhesion, invasion), gene expression of EMT markers (fibronectin, vimentin and ZEB2) as well as the expression levels of stem cell markers (Notch-1, OCT-4 and Nanog). Cell cycle was impaired in and the expression profiles of critical ECM components have been affected as well.
Discussion
Our data indicated that the studied breast cancer cells exhibited alterations in the expression levels of certain microRNAs that are implicated in the inhibition of cancer progression and the retention of EMT (i.e. miR-10b, miR-200b and miR-145), depending on the presence of ERα or ERβ and that these microRNAs significantly regulate breast cancer cell behavior, EMT process and ECM composition. These novel results suggest that ERs and EGFR are of crucial importance for the expression of ECM molecules and cell phenotype, highlighting their involvement in the behavior of cancer cells involving in cancer progression and along with targeting of ER-mediated microRNAs, might provide a potential target for advanced therapeutic approaches in breast cancer.

New therapeutic targets in genetic skeletal diseases

Mike Briggs
University of Newcastle

Genetic skeletal diseases (GSDs) are a diverse and complex group rare genetic conditions that affect the development of the skeleton. Although individually rare, as a group of related diseases, GSDs have an overall prevalence of at least 1 per 4,000 children. Remarkable progress has been made in identifying the genetic basis of GSDs and in key disease mechanisms. For example, we have previously established that endoplasmic reticulum (ER) stress, induced in chondrocytes as a result of accumulated misfolded mutant protein, is the primary cause of growth plate dysplasia and reduced bone growth in a broad group of GSDs. Moreover, genetic deletion of the Xbp1 branch of the unfolded protein response (UPR) increases disease severity, thus confirming the importance of ER stress and UPR in disease progression and identifying a novel therapeutic avenue.
In summary, the extensive clinical variability and genetic heterogeneity of GSDs renders this broad group of rare diseases a bench to bedside challenge. However, targeting ER stress through drug repurposing is potentially cheaper and faster than traditional drug discovery.

Workshop 7: The Immunology/Matrix Interface

Small leucine-rich proteoglycan in inflammation: two sides of the coin

Liliana Schaefer
Institute of Pharmacology and Toxicology, Goethe-University, Frankfurt, Germany

Biglycan, a prototype extracellular matrix-derived damage-associated molecular pattern, mediates sterile inflammation in macrophages through Toll-like receptor (TLR) 2 and/or TLR4-dependent signaling pathways. Recently we discovered that soluble biglycan is a novel high-affinity ligand for CD14, a well-known GPI-anchored co-receptor for TLRs. CD14 is required for all biglycan-mediated TLR2/4 dependent inflammatory signaling pathways in macrophages. By binding to CD14 and choosing different TLR signaling branches, biglycan induced TNF-〈 and CCL2 via TLR2/4, HSP70 through TLR2, and CCL5 via TLR4. Mechanistically, biglycan evoked phosphorylation and subsequent nuclear translocation of p38, p44/42, and NF-┤B, and these effects were due to a specific, high-affinity interaction between biglycan protein core and CD14. In a mouse model of renal ischemia/reperfusion injury lack of *Cd14* prevented biglycan-mediated cytokine expression, recruitment of macrophages, M1 macrophage polarization as well as mitigated the tubular damage and serum creatinine levels, thereby improving renal function.

Regulation of Matrix by Type 2 cytokines: Learning from Helminths

Judith Allen
WCCMR, University of Manchester

Helminth infection is strongly associated with the induction of a type 2 immune response, characterized by the production of the cytokines IL-4 and IL-13, both of which signal through the IL-4 receptor alpha. Type 2 immunity is essential for control of helminth infection, in part by repairing the damage caused by tissue migrating parasites. Macrophages activated by IL-4 and IL-13 exhibit a very distinct protein expression profile strongly associated with tissue injury and repair. The most abundant protein secreted by these macrophages is YM1, a molecule that binds extra cellular matrix but whose function is unknown. Our data suggest that YM1, in combination with other proteins induced by type 2 cytokines, contribute to repair by altering the dynamics and quality of collagen deposition.

Workshop 8: Matrix and Mechanobiology

Mechanobiology of Extracellular Matrix Fibers in vitro and in vivo

Viola Vogel
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Major transformation of extracellular matrix (ECM) composition, architecture and of its mechanical properties accompany inflammatory disease and cancer progression, yet little is known how this affects ECM-imposed outside-in cell signaling. As the ECM acts as reservoir for a plethora of growth factors and cytokines some of which are regulated by changing ECM fiber strain, gaining knowledge on the mechanical strain of ECM protein fibers in health and disease is urgently needed. Nothing is known so far about ECM fiber tension in healthy organs and during pathological transformations due to the previous lack of probes that can sense the tension of ECM fibers. Novel insights will be discussed which we obtained using our recently developed mechanosensitive peptide probes (FnBPA5) which specifically binds to relaxed, but not to stretched fibronectin (Fn) fibers, and how such peptides can be utilized for theranostic applications. Recapitulating tissue growth processes in microtissue platforms gave further insights into mechanisms by which ECM fiber tension might affect the fibroblast to myofibroblast transition.

Cellular contractility controlled by cell-ECM interactions sculpts organs and tissues

Carmina Santa Cruz Mateos, Andrea Valencia Expósito, Gokul Kannan, Isabel Palacios, Acaimo González Reyes, María D. Martín-Bermudo.

Centro Andaluz de Biología del Desarrollo, Spain

During morphogenesis, epithelial sheets, architectural units of most organs and tissues, are folded and reshaped to generate the 3D structure of organs. This is accomplished by changes on cell adhesion and actomyosin contractility. However, it is still unclear how the balance between adhesion and contractility is regulated during embryogenesis to allow the remodeling of tissues while maintaining their integrity. In recent years, E-cadherin junctions have emerged as centers where adhesion and contractility are integrated. In our lab, using the *Drosophila* follicular epithelium as model system, we have discovered that cell-ECM interactions mediated by integrins are also key regulators of the actomyosin contractile forces underlying epithelia morphogenesis. We have found that changes in the shape of follicle cells, underlying egg elongation, require a combination of surface tension generated by integrins at the membrane and intracellular force/tension generated by the actomyosin cytoskeleton. We are currently studying the biophysical properties of this interplay.

Plenary Session 4

BSMB Fell-Muir Award Lecture 2018

Gene cloning to clinical trials - the trials and tribulations of a life with collagen

Ray Boot-Handford
Wellcome Centre for Cell-Matrix Research, The University of Manchester, UK

My labs association with type X collagen began in the late 1980's with the successful cloning of the bovine cDNA and subsequently the full length sequence for human in a collaboration with Mike Grant upon my return to Manchester from the USA. Subsequently, we conducted extensive genetic screening and identified a number of the earliest cases of mutations in the COL10A1 gene causing metaphyseal chondrodysplasia type Schmid (MCDS). Later studies focused on generating and characterising mouse models of MCDS and we were able to demonstrate that increased ER stress was central to the disease mechanism in that stimulating ER stress in hypertrophic chondrocytes was sufficient to generate am MCDS-like phenotype in vivo. Most recently, we have been investigating ways of reducing ER stress in vivo and demonstrated that reducing the stress ameliorates the disease phenotype. Indeed, treatment of 2 MCDS mouse lines with carbamazepine (CBZ) significantly reduces disease severity and CBZ has now entered an EU-funded clinical trial for the treatment of MCDS.

Dick Heinegård Young Investigator Award Session

Modulating the physico-mechanical properties of basement membranes through the extracellular-matrix protein netrin-4

Raphael Reuten[1], Denise Nikodemus[1], Carina Prein[2], Ernst Pöschl[3], Martin Ehrbar[4], Peter Yurchenco[5], Jörg Stetefeld[6], Felix Bock[7], Thomas Langmann[8], Manuel Koch[9,10], Hauke Clausen-Schaumann[2], Janine Terra Erler[1]

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[9]*Institute for Dental Research and Oral Musculoskeletal Biology, Medical Faculty, University of Cologne, Joseph-Stelzmann-Strasse 52, Cologne 50931, Germany.*
[10]*Center for Biochemistry, Medical Faculty, University of Cologne, Joseph-Stelzmann-Strasse 52, Cologne 50931, Germany.*

The basement membrane (BM) is a specialized extracellular matrix, which serves as a physical barrier, supports tissue structures, and provides growth factors as well as cell attachment sites. BMs underlie the epithelium, endothelium, fat cells, Schwann cells, and muscle cells. Collagen IV and different laminin heterotrimers shape the core of the BM.

Laminins have an N-terminal globular domain on each of their short arms and assemble into a network through interaction with integrins on the cell surface. The secreted extracellular-matrix protein netrin-4 exhibits homology to the N-terminal domains of the laminin ® chain short arm. The majority of mechanistic studies of netrin-4 have been focused on an assumed interaction with canonical netrin receptors DCC/neogenin or UNC5B. However, we show that in contrast to netrin-1, netrin-4 does not bind directly to canonical netrin receptors. Instead, we link the high-affinity binding of netrin-4 to the N-terminal domain of the laminin © 1 chain to its anti-angiogenic as well as anti-tumorigenic potential. Here, netrin-4 disrupts the laminin network within the BM through decomposition of the laminin ⟨, ®, and © ternary node complex. Our study unveils netrin-4 as a non-enzymatic extracellular matrix protein actively disrupting pre-existing laminin network structures within BMs. These data not only yield a precise mechanism of netrin-4 function but also highlight novel therapeutic opportunities.

Mechanical properties of collagen fibrils in tendon

Brüggebusch Svensson
Institute of Sports Medicine, Copenhagen, Denmark

Tendon connective tissues are made of strong nanoscopic threads called collagen fibrils that are responsible for the mechanical integrity of the tissue. In spite of their important role, the mechanical properties of these fibrils are largely unexplored because their nanoscale dimensions present a technical challenge for such measurements. Using atomic force microscopy we have measured mechanical properties on fibrils from both human and rat tendons.

One important finding was that individual fibrils have viscoelastic behavior (stress relaxation). This is important because at the macroscopic level, viscoelasticity has usually been attributed to sliding of fibrils, but with this result macroscopic viscoelasticity can also be explained in part by viscoelastic relaxation of individual fibrils without sliding. We also found that the elastic modulus of the fibrils were fairly close to the properties of the macroscopic tendon in vivo, further indicating that only limited fibril sliding occurs under normal tendon loading. The length of collagen fibrils has been a longstanding question and analyzing the tendon microstructure by 3D reconstruction of electron microscopy images we found evidence that the majority of collagen fibrils in mature human tendons are continuous. This indicates that force transmission between fibrils may only play a small role in tendon mechanical function, in agreement with the limited sliding suggested by the mechanical results. Finally we have looked into the influence of biochemical cross-links and have found that highly cross-linked tissues have a distinct mechanical response with little effect at low strains but substantially increased strength and stiffness at high strains. Our most recent data suggest that a type of cross-link, which accumulates over time on long lived collagen due to reactions with sugars (advanced glycation end-products), may be the most important contributor to this mechanical behavior.

In the future we plan to investigate the influence of aging in humans, as well as the effects of different connective tissue affecting diseases in both human and animal models. *René*

Hydrogel-based delivery of antiMir-221 enhances cartilage matrix production by endogenous cells: Towards microRNA therapy for cartilage repair

Andrea Lolli[1], Kavitha Sivasubramaniyan[1], Maria L. Vainieri[2], Ezequiel Wexselblatt[3], Avner Yayon[3], Gerjo J.V.M. van Osch[1,4]

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Strategies for endogenous cartilage repair aim to recruit joint-resident stem cells to injuries and guide their chondrogenic differentiation. Previously, we showed that silencing microRNA(miR)-221 in human mesenchymal stem cells (hMSCs) *in vitro* strongly enhanced their cartilage matrix production *in vivo*. We here aimed to develop a fibrin/hyaluronan (FB/HA) hydrogel delivery system to inhibit miR-221 in endogenous stem cells in a cartilage defect, for guiding endogenous cartilage repair.

In vitro invasion of FB/HA by fluorescently labelled bone marrow hMSCs was assessed by confocal imaging. Chondrogenesis was confirmed by qRT-PCR and histology. FB/HA was loaded with fluorescent LNA- antimiR-221/lipofectamine complexes and the cumulative release was monitored over 14 days. FB/HA- mediated transfection of antimiR-221 into hMSCs and silencing of miR-221 were determined by flow cytometry and qRT-PCR analysis, respectively. An *in vivo* model of cartilage defect consisting of bovine osteochondral plugs implanted subcutaneously in athymic mice was employed to assess endogenous cell invasion and cartilage matrix production within FB/HA.

When incubated with FB/HA, hMSCs easily attached to its surface and infiltrated the hydrogel within 7 days. FB/HA supported *in vitro* hMSCs chondrogenesis, as shown by collagen II and aggrecan expression, and glycosaminoglycan production. *In vivo*, FB/HA demonstrated abundant endogenous cell invasion after 4 weeks. However, only limited cartilage matrix production with signs of undesired hypertrophic maturation was observed, indicating that further cues are needed to guide optimal cartilage repair. Thus, we loaded FB/HA with antimiR-221/lipofectamine complexes. FB/HA strongly retained antimiR-221 over time, with a minimal release of the inhibitor over 14 days (12,7%). Culture of hMSCs in antimiR-221 loaded FB/HA led to highly effective hMSCs transfection (~80%) and strong silencing of miR-221 within 7 days (≥95% for antimiR-221 ≥100nM). FB/HA loaded with antimiR-221 significantly enhanced production of cartilage matrix by endogenous cells *in vivo*. Interestingly, the newly-formed matrix did not show signs of undesired hypertrophic maturation.

By functionalizing FB/HA with antimiR-221, we developed a novel hydrogel delivery system that mediates knockdown of the anti-chondrogenic miR-221 *in situ*, strongly improving cartilage matrix repair by resident MSCs. Our work provides a proof-of-concept and a versatile technology for the manipulation of microRNAs to guide endogenous cartilage repair.

Regulation of tissue homeostasis by the tendon clock

Ching-Yan Chloé Yeung
Institute of Sports Medicine, Copenhagen, Denmark

Majority of the work was performed in the lab of Karl Kadler in collaboration with Qing-Jun Meng at Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine and Health, University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom. My current address where I am continuing my work is Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, Building 8, Nielsine Nielsens Vej 11, Copenhagen NV 2400, Denmark.

Circadian rhythm allows life on earth to coordinate appropriate physiology and behaviour with the 24-hour day. The molecular timekeeper, driven by self-oscillating BMAL1/CLOCK heterodimer transcription factors, is present in our peripheral tissues and regulates tissue-specific biological processes, including matrix homeostasis. Tendon's ability to transmit high tensile forces from muscle to bone is attributed to its highly organised collagen-rich matrix, and disruption to matrix homeostasis could lead to the development of tendinopathies.

We demonstrated that the clock mechanism in tendon regulates a 24-hourly rhythmic expression of over 700 genes and observed spontaneous calcification in tendon of young *Clock* mutant mice[1]. Importantly, tendon calcification was also observed in aged wild type mice, which was concomitant with a dampened tendon circadian rhythm that out-of-sync in phase to the central clock and loss of time- dependent expression of clock-controlled genes. Tendon's most rhythmic transcript encodes matrix metalloproteinase 14 (MMP14). MMP14 cleavage of fibronectin is essential for collagen fibril release from the cell membrane to allow matrix expansion during tendon development[2]. We sought to understand how clock-controlled *Mmp14* regulates matrix homeostasis in postnatal tendon.

In tendons of tendon-specific *Bmal1* knockout mice we observed loss of time-dependent *Mmp14* expression, fibrosis and impaired mechanical properties. We established that synchronised primary mouse tenocytes in culture have rhythmic expression MMP14 and flotillin-1, a lipid raft-associated cargo-sorting and exosome-associated protein. Western blotting and immuno-electron microscopy analyses corroborated the enrichment of MMP14 in released exosomes isolated by ultracentrifugation and sucrose gradient fractionation. MMP14 enrichment in exosomes was modulated by siRNA-mediated knockdown of *Flot1*. Tamoxifen-inducible knockout of *Mmp14* in fibroblasts of adult mice was sufficient to cause massive tendon fibrosis and transmission electron microscopy analysis revealed an accumulation of narrow (50-nm) diameter collagen fibrils. Proteomic analysis of these *Mmp14* KO tendons at two time points confirmed loss of time-dependent abundance of fibronectin.

Together, our data strongly suggest that circadianly-regulated *Mmp14* expression in tendon forms a major pathway for matrix turnover and we hypothesise that packaging into extracellular vesicles, via clock-regulated flotillin-1, enables MMP14 to efficiently mediate a tissue-wide effect. Given its importance, decline of the tendon clock in ageing could help explain the increased prevalence of tendinopathies with age.

Crystal structure of dermatan sulfate epimerase 1 and investigations into its interaction with dermatan 4-sulfotransferase 1

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The amount and distribution of iduronic acid (IdoA) and sulfate groups are the most important structural requirements for the numerous functions of chondroitin/dermatan sulfate (CS/DS), such as cell migration, collagen fibrillogenesis, interaction with growth factors and cytokines, and control of coagulation. Four biosynthetic enzymes are of central importance; dermatan sulfate epimerase 1 and 2 (DS-epi1/2), dermatan 4-sulfotransferase 1 (D4ST1), uronyl 2-sulfotransferase (UST) and N-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase (CHST15).

We have previously shown that recombinant DS-epi1 alone is a partially processive enzyme forming short sequences of adjacent IdoA which slides from the reducing towards the non-reducing terminal of chondroitin. The addition of recombinant D4ST1 and PAPS gave full processive capability to the reaction, resulting in formation of long stretches of IdoA-GalNAc-4S structures. Further addition of recombinant UST or CHST15 generated up to 70% IdoA-2S-GalNac-4S or 90% IdoA-GalNac-4S,6S, respectively. Finally, modification of IdoA-2S-GalNac-4S by CHST15 generated 2,4,6 sulfated dermatan sulfate.

In order to understand the formation of IdoA on a molecular level, we crystallized and solved the 3D structure of DS-epi1 with a resolution of 2.4 Å. The structure gave information concerning N-glycosylations, active site residues and structural explanations for evolutionary conserved domains among species.

To understand how the different modifications are accomplished, we investigated the organization of the biosynthetic complexes in Golgi using FRET methodology. DS-epi1, DS-epi2 and D4ST1 were found as homodimers. In addition, DS-epi1 interacted with DS-epi2 and D4ST1, but DS-epi2 and D4ST1 did not interact. After chemical crosslinking of recombinant proteins, followed by mass spectrometry analysis and Rosetta modeling, we could define the overall 3D structure of the heterooligomeric complex of DS-epi1/D4ST1. Thus, the formation of IdoA-containing motifs in CS/DS depends on interactions between the biosynthetic enzymes to form biologically active, sequence- and size-defined structures.

Plenary Session 5

Biosynthesis and function of (honorary) glycosaminoglycans: insights from structural studies

Erhard Hohenester,
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Glcyosaminoglycans (GAGs) are long unbranched polysaccharide chains that are attached to a number of secreted and cell surface proteins. The negatively charged GAGs have important functions in the extracellular matrix and serve as essential co-receptors for many morphogens and growth factors. I will discuss how the first enzyme in GAG biosynthesis, peptide *O*-xylosyltransferase, selects specific sites within the core proteins for GAG modification. Recently, the laminin-binding modification of the transmembrane protein dystroglycan was revealed to be a GAG-like polymer of glucuronic acid-xylose units; absence of this modification causes severe muscular dystrophyies with brain abnormalities. I will present our recent results on the laminin-dystroglycan interaction.

Poster Abstracts (including selected talks, STs)

Workshop 1: Stem Cells and Matrix Engineering

P1, The Effect of Extracellular Matrix Stiffness on Pancreatic Cancer Stem Cells in a 3D Matrix Model

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Introduction
Pancreatic cancer is a CR-UK cancer of unmet need, primarily due to delayed diagnosis and ineffective treatments. The marked fibrosis and dense stroma is thought to serve as a barrier to anticancer drugs in pancreatic ductal adenocarcinoma (PDAC). As the rigid matrix forces and counteracts the cancer cells, the stiffened extracellular matrix (ECM) can affect cellular behavior and promote tumour development. Cancer stem cells (CSCs), a small percentage of the cancer cell population, are characterized by their multipotency and the ability to initiate cancer, propagate metastases, and are resistant to chemotherapeutic drugs. We hypothesized that the stiffened ECM may support CSCs, and therefore promote PDAC development. Therefore, we employed a self-assembling peptide amphiphile (PA) that exhibit tuneable rigidity as a 3D matrix model to investigate the relationship between stiffness ECM and CSCs in pancreatic cancer progression.

Materials and Methods
A new PA was designed in our group, and different stiffness of PA gel was obtained by introducing different concentrations of $CaCl_2$. AFM was applied to measure the stiffness of pancreatic cancer patient derived xenografts (PDX); confocal imaging and q-PCR were used to examine cell viability, EMT and CSC gene expression.

Results
The stiffness range of PDX is about 1-20 kPa; the corresponding stiffness PA gel was formed by 0.01-0.1M $CaCl_2$. Compared to 2D cell culture, PDAC in PA hydrogel showed good cell viability within 21 days. As the stiffness of hydrogel increased, the EMT and CSC related gene expression in PDAC increased at mRNA level; CSC gene expression elevated more once hyaluronan was introduced into stiffer hydrogel; PDAC encapsulated in stiffer hydrogel showed the high chemotherapy resistance.

Discussion
These data show that stiffer ECM stimulates EMT and CSC related gene expression, and decreases antineoplastic drug sensitivity in PDAC. Many studies reported that cells respond to force on integrin-mediated adhesions by remodeling the ECM through upregulating FAK and PI3K, and activating Rock to increase actomyosin-mediated cellular tension. Therefore, future studies will focus on FAK/PI3K/Rac1 or Rock signal pathway to investigate the relationship between stiffness matrix and pancreatic CSCs. This study highlights the potential for targeting CSCs via mechanical property of tumour microenvironment as promising therapeutic strategy that inhibit tumour progression.

P2, SIRT1 ACTIVATION IN ESC DERIVED PRECHONDROCYTES PROMOTES CARTILAGE ECM EXPRESSION

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Introduction
Regulation of gene expression and transcription factors by epigenetic factors is essential for successful differentiation. SIRT1, is a histone deacytalase enzyme, able to bind and deacetylate the main chondrogenic factor SOX9. Indeed, osteoarthritic and dedifferentiating primary chondrocytes display decreased SIRT1 protein expression. The aim of this study is to identify the role and activity of SIRT1 during the differentiation of human pluripotent stem cells hPSCs to chondrocytes and its impact of extracellular matrix expression.

Materials and Methods
hPSCs were differentiated to prechondrogenic cells using a 2D 14-day defined differentiation protocol. At 14-days, cells were pelleted and cultured for an additional 14-day period in 3D pellet culture, with SIRT1 activator (SRT1720) or inhibitor (EX527). Additionally, TC28a2 immortalized juvenile chondrocytes were cultured in pellet culture, with SIRT1 activator or inhibitor. QRT-PCR and protein expression was used to assess chondrogenic output. ChIP-PCR was used to determine chromatin binding of SIRT1 under different conditions. Histological assessment was performed to determine pellet structure.

Results
Result show no beneficial effect of activation or inhibition of SIRT1 during the 2D chondrogenesis stage with no change to COL2A1 or ACAN gene expression. During 3D culture inhibition of SIRT1 caused no significant change in gene expression compared to control. Activation of SIRT1 in 3D led to significant increases in SOX5, ARID5B and ACAN, with significant decreases in COL1A1 and RUNX2 gene expression (Fig 1). Likewise, activation of SIRT1 in TC28a2 cells only led to an increase in ECM gene expression in 3D not 2D, in particular SOX5 and ACAN. This was supported by western blot analysis of ACAN which showed a 3.5-fold increase in activated cells. Overexpression of SIRT1 in TC28a2 cells did not result in an increase of ECM gene expression

Discussion
The results of this study indicate that SIRT1 expression and activity are important to PSC derived chondrocyte development, by being involved with a protein complex required for the transcription of chondrogenic genes.

P3, Identification of synovial fluid proteins that are associated with early Osteoarthritis treatment failure: the search for novel markers leads us back to Matrix Metalloproteinases (MMPs)

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Introduction

Autologous Chondrocyte Implantation (ACI) is a cell therapy used to treat cartilage defects and early osteoarthritis. During initial surgery (Stage I) healthy cartilage is harvested from the joint. Chondrocytes are isolated and culture expanded before being implanted into the defect site (Stage II). An alternative cell-based therapy uses patients' bone marrow (BM), which is collected without cartilage harvest at Stage I; subsequently culture expanded BM-mesenchymal stromal cells (MSCs) are implanted at Stage II. 20% of ACI treated patients demonstrate no clinical benefit. We aim to better understand why these individuals fail.

Materials and Methods

Two proteomic techniques (isobaric tag for relative and absolute quantitation and label-free quantitative liquid chromatography tandem mass spectrometry) were used to comprehensively profile the proteome of knee synovial fluid (SF) samples. 14 ACI responders' and 13 non-responders' SFs collected at Stages I and II were analysed. ACI response was determined by change in functional knee score at 12 months. Proteins showing differential levels were validated using Quantikine® immunoassays in this cohort and a further independent group of patients which included those treated with BM-MSCs (i.e. no cartilage harvest procedure).

Results

Proteomic analyses highlighted that MMPs 1&3 demonstrate increased abundance at Stage II compared to Stage I, only in the ACI failure group (MMP-1 increased 2.7 fold; MMP-3 increased 2.9 fold). These observations were validated by immunoassay (MMP-1, Stage I, 800 ± 889pg/ml, Stage II, 7741 ± 8065pg/ml, $p=0.006$; MMP-3, Stage I, 34 ± 17ng/ml, Stage II, 61 ± 17ng/ml, $p=0.002$, Mann-Whitney U test). In the independent cohort, MMP-1 (n=31) and MMP-3 (n=47) were increased at Stage II compared to Stage I, only in the patients who underwent a cartilage harvest procedure during Stage I ($p<0.0001$; Wilcoxon-matched pairs).

Discussion

This study suggests that there may be an acute catabolic response in the knee to the cartilage harvest procedure in ACI only in individuals who do not respond well clinically. Further study is required to determine
whether the cartilage harvest procedure itself is damaging in these patients or whether this response to cartilage injury is indicative of a poor innate capacity for cartilage repair.

P4, ST: Insulin-like growth factor binding protein (Igfbp6) is a cross-species transcriptomic tendon marker

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Introduction
Evaluation criteria of bioengineered tendon constructs are unclear partially due to the lack of specific markers of tendon development and differentiation. The aim of this study was to identify a panel of genes that exhibit clearly higher expression in tendon relative to cartilage and muscle and validate them across key model species utilised in tendon research.

Materials and Methods
Comprehensive gene expression profiling of rat tendon and cartilage was undertaken using two independent microarray platforms. Illumina RatRef v12 was used for analysing whole tissues while Affymetrix GeneST Rat for isolated primary tenocytes and chondrocytes. Processing of raw gene expression data and differential expression analysis was undertaken using software packages in R. Genes that demonstrated high correlation in expression levels across two studies were validated by qRT-PCR in whole rat tendon relative to cartilage and muscle. Five genes demonstrating the highest expression in validation experiment were selected for further evaluation by qRT-PCR across different musculoskeletal tissues in ovine and equine.

Results
Genes that demonstrated the highest tendon expression (log2 fold-change >1.5) in both microarray studies, relative to cartilage, included: *Tmnd*, *Serpinf1*, *Igfbp6*, *Cxcl13*, *Cpxm2*, *Mfap5* and *Aspn*. *Meox2*, *Mustn1*, *Thbs4*, *Thbs2*, and *Prrx1* demonstrated more variable expression between the two platforms. Genes showing higher expression in tendon were enriched for functional terms relating to 'developmental processes' and 'extracellular matrix'. In qRT-PCR analysis of rat musculoskeletal tissues, significantly higher expression in tendon was detected for *Cpxm2*, *Myoc*, *Mfap5*, *Serpf1* ($p<0.05$) and *Aspn*, *Ecm1*, *Igfbp6*, *Tnmd* and *Thbs4* ($p<0.001$). Only *Igfbp6* and *Tnmd* demonstrated significantly higher expression in the tendon of all species relative to cartilage and muscle.

Discussion
The initial pool of tendon gene markers, identified by unbiased transciptomic analysis of musculoskeletal tissues in rat, demonstrated high variability in other model species. Insulin-like growth factor binding protein 6 (*Igfbp6*) was identified as the only universal tendon marker, comparable with that previously recognised; tenomodulin (*Tnmd*). Altered expression of *Igfbp6* has been described previously in animal models of tendon injury and human fibroblasts affected by Duptryen's disease. *Igfbp6* may be considered a potential reference biomarker for evaluation of tendon physiological function and directed development of engineered tendon.

P5, Matrix-inspired biomaterials for cell phenotype control

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Introduction
Glycosaminoglycans (GAGs) and proteins within the extracellular matrix (ECM) regulate many cell processes including proliferation, differentiation and migration, with dysregulation of ECM composition and structure often associated with disease. There are currently a few, rare disorders linked to mutations in GAG-biosynthetic genes, such as Multiple Osteochondroma (MO), with tissue-specific phenotypes that are puzzling given the ubiquitous expression of the GAG they generate. However, complex *in vivo* and *in vitro* assay systems currently impede our ability to define the specific mechanistic basis of ECM regulation in development and disease, critical for understanding matrix control of cell behaviour. To address the need for defined culture environments with which to model the ECM, and ECM-related diseases, we will assess the impact of matrix components on directed stem cell differentiation using a synthetic self-assembling peptide hydrogel-based 3D culture method.

Materials and Methods
Human pluripotent stem cells (hPSCs) were cultured in 'naked' (non-functionalised) self-assembling octapeptide hydrogels under pluripotency-supporting (Essential 8 media) and differentiation-supporting (Essential 6 media for spontaneous differentiation and N2B27 media for neural differentiation) conditions. Immunocytochemistry was used to visualise protein and GAG expression throughout differentiation. Changes in mRNA levels of genes involved in pluripotency and differentiation were quantified using qRT-PCR. To create a disease model, induced pluripotent stem cells (iPSCs) were reprogrammed from MO patient material and CRISPR/Cas9 gene editing will be used to generate isogenic controls.

Results
Early data demonstrates continuous culture and differentiation of hPSCs in 'naked' unmodified hydrogels, in the absence of any exogenous matrix components (e.g. Matrigel, serum or co-culture). iPSCs generated from MO patients exhibit a normal karyotype and are able to differentiate into the three germ layers.

Discussion
Culturing and differentiating hPSCs in this peptide hydrogel system, under completely defined conditions, enables assessment of the impact of specific ECM additions in a fully controlled manner. The gels have tuneable rheological properties and can be functionalised with bespoke proteins or GAGs to mimic the tissue-specific properties of the ECM. Combining advanced genetic engineering with modern stem cell technology and a flexible 3D hydrogel culture system, patient-specific disease models will be created to study the impact of ECM on cell behaviour and disease development.

P6, Comparison of chondrogenic potential of bone marrow and umbilical cord blood derived mesenchymal stem cells in an attempt to improve cartilage tissue engineering strategies.

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Introduction
Cartilage tissue engineering and/or cell therapy offer new insights to cure articular disorders such as osteoarthritis. Mesenchymal stem cells (MSC) represent an attractive cell type in order to produce a hyaline cartilage substitute. This study aimed to compare the chondrogenic potential of equine umbilical cord blood (UCB) and bone marrow (BM) derived MSC.

Materials and Methods
BM and UCB MSC were isolated and then amplified in monolayer culture. We characterized MSC by assessing their proliferative and multipotency capacities, and the presence of cluster of differentiation characteristics of MSC. Then, we compared MSC at their undifferentiated basal state and after chondrogenic differentiation. Chondrogenesis was induced by culturing the cells in a type I/III collagen biomaterial with BMP-2 and TGF-ß1. To determine the best oxic condition, the chondrogenic differentiation was performed either in hypoxia and normoxia. To compare the MSC chondrogenic potential we evaluated the mRNA levels and protein amounts of several osteogenic (Osteocalcin/Runx2), hypertrophic (type X collagen), fibrocartilage (type I collagen) and chondrogenic markers (type II collagen). Furthermore, the extracellular matrix (ECM) composition/structure was analysed by immunohistochemistry.

Results
The MSC characterization allowed to determine that UCB and BM MSC have different multipotency capacities as well as proliferative abilities. At their undifferentiated basal status, UCB and BM MSC exhibited also differences in the expression of type I collagen and of osteogenic, chondrogenic, hypertrophic markers. Upon culture of the cells in a chondrogenic induction medium, both MSC were able to increase their chondrogenic markers expression and seemed to produce an ECM of better quality in hypoxia, although type I collagen remained expressed. Nevertheless, both MSC exhibited an upregulation of type I collagen synthesis. UCB MSC produced the highest protein amounts of type II collagen but also of type I collagen, whatever the oxic condition.

Discussion
Both BM and UCB MSC were able to produce a hyaline-like cartilage matrix upon a chondrogenic differentiation protocol whatever the oxic condition. However, considering that type I collagen is the major undesirable component in the *in vitro* cartilage neosynthesis, BM MSC seem to be the best MSC candidate for cartilage tissue engineering.

P7, ST: In vivo wound healing with an engineered matrix-rich living modular construct for stem cell delivery

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Introduction
Advanced therapeutic medicinal products for engineering cutaneous substitutes are an evolving option, aiming to improve wound healing outcomes by reducing wound healing closure time and delivering cells, medical compounds and biologics. Matrix – rich tissue equivalents can be fabricated in vitro by employing biophysical, biological and biochemical cues. Our work is focused on the production of matrix-rich implantable tissue equivalents. Herein, we investigated the effect of macromolecular crowding on the fabrication of a matrix rich tissue equivalent for a wound healing application.

Materials and Methods
A collagen-based film has been utilised for the fabrication of a modular, matrix-rich cell carrier (bone marrow stem cells) for wound healing. The structural, mechanical and thermal properties of the material were assessed with electronic microscopy, uniaxial mechanical testing and differential scanning calorimetry (DSC) respectively. For the enhancement of extracellular matrix deposition, a macromolecular crowding agent (Carrageenan) was utilised at all time points. Matrix deposition was assessed with immucytochemisty. A splinted wound healing model in athymic nude mice was utilised to assess wound healing in vivo. Wound healing closure ratio was assessed on day 3,7 and 14. Tissues were harvested 14 days post implantation for histology.

Results
Extracellular matrix deposition has been enhanced at all time points when carrageenan was used as a macromolecular crowding agent in the in vitro regime. Constructs grown in vitro in the presence of carrageenan, facilitate accelerated and improved wound healing in vivo when implanted in the athymic nude mouse model.

Discussion
Macromolecular crowding enhances matrix deposition in vitro, contributing to the *in vitro* maturation of implantable modular constructs which translates to better and faster wound healing *in vivo.*

P8, ST: Deriving Nucleus Pulposus-like Progenitors from MAP kinase Interference Coupled Chondrogenic Induction in Mesenchymal stem cells

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Introduction
Depletion of extracellular matrix producing nucleus pulposus (NP) cells in intervertebral disc (IVD) degeneration limits the self-repair ability *per se.* Adult NP progenitor cells expressing tyrosine kinase receptor 2 (Tie2) and disialoganglioside 2 (GD2) were identified with *in vitro* clonogenicity and *in vivo* self-renewal ability. By microarray transcriptomic analysis, we identified preferential expressions of *Cadherin 2* (*Cdh2*), *keratin 19* (*Krt19*) and *carbonic anhydrase 3* (*Car3*) in rat primitive NP cells (PNPCs) compared to chondrocytes. *In silico* analysis of signaling pathways suggested PNPCs exhibiting low TGF-β and MAP kinase activities. Mesenchymal stem cells (MSCs) were demonstrated to differentiate into NP-like cells via TGF-β stimulation. Here, we hypothesized that MAP kinase interference coupled chondrogenic induction (MICCI) of MSCs could differentiate into NP progenitor-like cells. To this end, we characterized protein expressions of defined molecular markers in Colonies Formation Units-Spherical (CFU-S) from human NP cells. Thereafter, we evaluated gene expressions of CFU-S molecular markers in MICCI of MSC micro-pellets.

Materials and Methods
CFU-S assay was accessed by seeding a single-cell suspension of 1 x 10^3 human NP cells / 1ml of methylcellulose medium (*Stem Cell Technologies*) for 14 days. Immunostaining of cyto-spun CFU-S were performed with (*Santa Cruz*): anti-Tie2, anti-Krt19, anti-Car3; (*abcam*): anti-Cdh2, anti-CD24, anti-type II collagen, anti-aggrecan; and (*BD biosciences*): anti-GD2 antibodies. TGF-β1-based chondrogenic induction of human bone marrow MSC micro-pellet culture (2 x 10^5 cells) were treated with or without 10 ng/ml inhibitors of MEK1/2 (PD98059) for 14 days. Quantitative PCR (QPCR) was performed using Taqman probes (*Thermo Fisher scientific*): *TEK (*HS00945746_m1); *CDH2* (HS00983056_m1); *AGC1* (HS00153936_m1); and normalized by *GAPDH* (HS02758991_g1).

Results
By immunostaining, previously reported markers: Tie2, GD2, aggrecan (AGC1) and type II collagen (COL2) were detected in human NP derived CFU-S. CDH2, but not for KRT19, CAR3 and CD24, were detected in CFU-S. QPCR showed a strong upregulation of *TEK1*, *CDH2* and *AGC1* in MEK1/2 inhibited MSC micro-pellets after Day 7 and onwards. Enhanced proteoglycan deposition was also detected in MEK1/2 inhibited MSC micro-pellets.

Discussion
Tie2, GD2, COL2, AGC1 and CDH2 were expressed in human NP derived CFU-S and might represent as molecular markers of NP progenitors. MICCI of MSC micro-pellets could induce CFU-S molecular markers with enhance proteoglycan deposition. This study provides a simple, yet effective protocol in the generation of NP progenitors from MSCs for IVD engineering.

P9, ST: Identification and *in vitro* screening of osteogenic metabolites through supplement-free nanovibration-driven mesenchymal stem cell differentiation

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Introduction

There is a need for the development of effective tissue engineered approaches to produce bone. In the laboratory, these approaches typically involve osteogenic differentiation of mesenchymal stem cells (MSCs) through media supplementation. We recently developed a supplement-free osteogenic differentiation protocol through nanovibrational-stimulation of MSCs[1]. Here, we hypothesised that nanovibrational differentiation of MSCs would allow metabolomic analysis of differentiation without confounding exogenous media supplements. We aimed to investigate MSC nanovibration-driven osteogenesis in 2D and 3D cultures, identify key osteogenic metabolites and metabolomic processes and investigate their osteogenic potential by supplementing these pathways *in vitro*.

Materials and Methods

Human MSCs were cultured in standard tissue culture well plates (2D) or in type I collagen gels (3D) and cultured over 28 days in three groups- nanovibrational stimulation, osteogenic media (dexamethasone) and MSC expansion media. Differentiation was tracked through changes in gene expression (qPCR), protein expression (immunofluorescent staining (IFS)). At key points, cell metabolomic analysis was performed (LC-MS; ZIC-pHILIC). We selected the most promising metabolite during osteogenic differentiation. This was synthesised along with several chemical analogs. The osteogenic potential of these metabolites was then investigated through gene and protein expression following supplementation to 2D and 3D MSC cultures.

Results

Nanovibration upregulated key osteogenic genes in both 2D and 3D cultures comparably to osteogenic media, including early upregulation of RUNX2 (2D x14.5, $p<0.05$; 3D x11.5, $p<0.05$) followed by maturation marker osteopontin (2D x19, $p<0.05$; 3D x7.2, $p<0.05$). Corresponding increases in osteogenic proteins were also observed. Metabolomic analysis identified several key networks, with cholesterol sulphate (CS) identified as a promising metabolite target. When CS and several analogs were supplemented at 1 μM to 2D and 3D cultures they induced osteogenic gene and protein expression, comparably to osteogenic media, while having less off target effects. In particular, fludrocortisone and fludrocortisone acetate significantly increased osteogenic marker expression, even versus osteogenic media

Discussion

Nanovibration is an exciting tool for the supplement-free study of MSC osteogenic differentiation, while this work also validates a targeted metabolite supplementation approach for controlling cell fate decisions, which may prove cheaper and more specific than conventional approaches.

P10, Investigating the shared molecular basis of diseases of glycosaminoglycan synthesis and degradation

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Introduction
Glycosaminoglycans (GAGs), including heparan sulphate (HS), are involved in the regulation of many essential processes such as cell survival, migration, differentiation and adhesion via regulation of multiple interacting signalling pathways. Mutations in genes for key enzymes in the HS synthetic and degradative pathways such as IDUA, EXT1 and NDST1, lead to severe pathologies, including Mucopolysaccharidosis type I (MPSI), Multiple Osteochondromas (MO) and Autosomal Recessive Intellectual Disability (ARID), respectively. Although the phenotypes of these disease are very different, they share a characteristic accumulation of abnormal GAG structures which then impact on the GAG-regulated pathways introduced above. We are creating induced pluripotent stem cell (iPSC)-based disease models, either by reprogramming patient tissue or by CRISPR-Cas9 knock-out. Critically, as these disorders are associated with altered pericellular matrix deposition, we will use a defined, fully synthetic peptide hydrogel system, free from exogenous GAG, to study 3D iPSC growth and differentiation.

Materials and Methods
Human iPSCs are cultured in non-functionalised self-assembling peptide hydrogels in Essential 8 (E8) and E6 media. Immunocytochemistry and flow cytometry will be used to characterise the GAG profiles of the different cell lines. N2B27 media will be used to direct neural differentiation.Human iPSCs will be transfected with wild-type CRISPR Cas9 constructs to knock out EXT1, NDST1 and IDUA, creating both heterozygous and homozygous cell lines.

Results
Successful culture and differentiation of human wild type iPSCs in the peptide hydrogels has been achieved, without the need for serum, other matrix addition or co-culture. Immunocytochemical staining of GAG chains in 2D and 3D culture using specific antibodies has proved to be an effective method to visualise the GAGs deposited by the cells. Guide and targeting plasmids for CRISPR Cas9 gene editing have been constructed ready for transfection.

Discussion
Culturing cells in a defined, GAG-free 3D system allows the study of disease-typical GAGs secreted by cells engineered to model matrix-relevant genetic diseases. The system also allows for the addition of defined ECM components to test their impact on cell behaviour. We therefore aim to develop a suite of models to enable us to study the potential shared mechanistic dysregulation of signalling pathways between diseases of GAG synthesis and GAG degradation.

P11, Nanotopography of substrates directs the deposition of organised fibrillar collagen by corneal stromal cells

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Introduction
The corneal stroma constitutes 90% of the cornea. It consists of around 200 collagen lamellae, each oriented roughly orthogonally to neighbouring lamellae. Within each of these are highly aligned collagen fibrils and interspersed between them are keratocytes, which are responsible for maintaining transparency. Corneal scarring is a leading cause of blindness globally. A shortage of donors, with only 1 cornea available for every 70 corneas needed, has led to a tissue engineering solution being required. Furthermore, current *in vitro* models of the stroma focus on using a collagen gel or layers of fibroblasts that fail to replicate the microstructure. By being able to more faithfully recapitulate the stroma we aim to develop an *in vitro* model to better explore the mechanisms of collagen alignment.

Materials and Methods
Cells isolated from human corneo-scleral rims were cultured on coverslips with polytetrafluoroethylene (PTFE) nanofibres. Cell Tracker and a collagen probe (CNA35) were added to culture media and imaged for up to 3 days to observe the live cell deposition of collagen. Separate samples were cultured for several weeks to form a cell layer and the alignment of the extracellular matrix (ECM) was analysed using OrientationJ, an ImageJ plugin.

Results
Collagen fibres could be visualised using a FITC-labelled collagen probe (CNA35) whilst cells were highlighted using Cell Tracker. OrientationJ analysis of collagen fibres showed that when the cells were cultured on PTFE nanofibres around 73% of the fibrils were aligned within ± 10° of the dominant direction, compared to just 44% when cultured on a control substrate.

Discussion
It has been well documented that cells respond to topographical cues and this has been exploited in a variety of applications. The PTFE nanofibres provided the topographical cues for cells, and thus collagen, to align along. Interestingly, we observed that as stromal cells stratified they maintained their alignment but rotated by 37°. Literature suggests that cell layers that maintain organisation have improved tissue functionality. By manipulating cell sheets, we hypothesise that we'll be able to produce multiple cell layers that can be organised to recapitulate the native cornea for use as either a graft alternative or an *in vitro* model.

P12, Synthetic photoreceptor engineering for optogenetic control of TGFβ signalling

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Introduction
The transforming growth factor-β (TGFβ) superfamily (including bone morphogenetic proteins – BMPs) are a family of signalling molecules crucial in chondrogenic development. Current chondrogenic directed differentiation protocols (DDPs) of human pluripotent stem cells (hPSCs) rely upon timed supplementation of growth factors. However, this may lead to poor differentiation reproducibly and quality because of batch-to-batch variation. Due to the essential role of the TGFβ-family during chondrogenesis, precise control of the signalling pathway may enable refinement of differentiation and improvement of ECM production. This aim of this project is to utilise the novel technology of optogenetics to render the activation of the BMP-pathway light-sensitive; enabling fine-tuning of signalling during chondrogenesis.

Materials and Methods
Differentiation of MAN13 human embryonic stem cells (hESCs) towards chondrocytes was performed following an established protocol. Differentiation was evaluated through RT-qPCR gene expression analysis of key chondrogenic markers. Optogenetic BMP-like receptors were generated through PCR and 'NeB HiFi Assembly' cloning and inserted into a doxycycline inducible vector. Chondrosarcoma SW1353 cells were transduced with lentiviral particles containing a BMP-like SMAD1/5/8 transcriptional response element (BRE) reporter along with optogenetic BMP Type I and II receptors. Cells were dosed with doxycycline before flashing with blue light and SMAD1/5/8 transcriptional activity was measured through nano-luciferase production.

Results
An established chondrogenic DDP resulted in significant upregulation of chondrogenic-associated gene expression SOX9, SOX5 and COL2a1 after 14 days in 2D monolayer culture. Optogenetic BMP-like receptors were successfully generated and expressed by transduced cells through stimulation with doxycycline. Activation of optogenetic receptors with blue light resulted in nano-luciferase production; indicating stimulation of the BRE reporter by transcriptionally active BMP-like SMAD1/5/8.

Discussion
Findings shown here demonstrate the applicability of optogenetics for control of BMP signalling and a framework for future approaches. Light-stimulated dimerisation of Type I and II BMP-like receptors appeared sufficient to stimulate SMAD1/5/8 activity and variable light dosage should enable fine-tuning of signal transduction. Incorporation of light-controlled BMP signalling in chondrogenic directed differentiation of hESCs may reveal rate-limiting steps and enable improvement of chondrogenic differentiation in the future.

P13, Manipulating co-regulators of RUNX2 and SOX9 to enhance the chondrogenic potential of chondrogenic progenitor cells in osteoarthritis

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Introduction
The regeneration of diseased hyaline cartilage continues to be a great challenge, mainly because degeneration overtaxes the tissue's self-renewal capacity. Recently, we demonstrated that repair tissue from human articular cartilage during the late stages of osteoarthritis harbors a unique progenitor cell population, termed chondrogenic progenitor cells (CPCs). Down-regulation of the osteogenic transcription factor RUNX2 enhanced the expression of the chondrogenic transcription factor SOX9. This, in turn, increased the matrix synthesis potential of the CPCs without altering their migratory capacity. We now present unpublished data on the role of co-regulators of SOX9 and RUNX2 to enhance the chondrogenic potential of CPCs.

Materials and Methods
Candidate molecules for example, RAB5C, YWHAE or DDX5, identified in an elaborated knock-down and pull down experiment have been overexpressed or knocked-out via CRISPR/Cas9. The effect on the chondrogenic potential was investigated by qPCR, Western blot and immunohistochemistry.

Results
The manipulation of co-regulators of SOX9 and RUNX2 enhances the chondrogenic potential of CPCs. We observed altered expression levels of RUNX2, SOX9 as well as collagen type I and type II in 2D and 3D cell culture.

Discussion
Our data indicate that we can successfully manipulate the chondrogenic potential of CPCs in vitro. Further research will focus on the improvement of the cartilage composition in vivo, by transplantation of suitable candidate cell lines into the mouse model. This will lead to the identification of new signalling pathways as attractive targets for future OA therapy.

P14, Altered Matrix Adhesion, Impaired Function and Mitochondrial Hyperactivity in Endothelial Colony Forming Cells Isolated from Patients with Diabetic Foot Ulcers

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Introduction

Patients with diabetes have impaired endothelial colony forming cell (ECFC or endothelial progenitor cell) function leading to poor vascular endothelial repair, potentially contributing to diabetic foot ulceration, a major health and economic burden. The objective was to characterise the functional deficit in these cells in order to understand the pathogenic mechanism and identify novel therapeutic targets.

Materials and Methods

ECFCs were harvested from the peripheral blood of healthy controls and patients with diabetes and neuroischaemic foot ulcers using cell culture techniques. Functional and biochemical analyses of the migratory, angiogenic and metabolic activity were performed. In addition, a neotissue array was used to compare ECFC binding and growth to different matrix ligands (RGD, DGEA, IKVAV, YIGSR, VAPG) and combinations of ligands, including soft and fibrous matrices to represent distinct environments.

Results

Neuroischaemic ECFCs take longer to form colonies on isolation, have impaired nitric oxide production (46% of control) and a reduced migratory response to SDF-1 (77% of control), decreased scratch closure (87% of control) and compromised tube formation (38% of control in the matrigel assay compared to healthy ECFCs). Seahorse extracellular flux analysis of metabolic function identified no change in glycolysis, but mitochondrial function and maximal oxygen consumption was increased two fold in the neuroischaemic ECFCs *vs* healthy ECFCs. The neotissue array revealed a decrease in binding of neuroischaemic and control ECFCs to stiff matrix compared to normal physiological stiffness by between a half and two thirds over 24 hours. The neuroischaemic ECFCs bound less, and to a reduced repertoire of matrix peptides compared to controls, suggesting a diminished binding capacity. Furthermore, in long-term culture the viability of neuroischaemic ECFCs was reduced compared to control cells.

Discussion

This study is the first to describe the defect in matrix adhesion and metabolic changes in neuroischaemic diabetic ECFCs, which may contribute to impaired endothelial repair observed *in vivo*. Further work to characterise the mechanism of this binding deficiency will allow us to develop an improved model of disease *in vitro,* leading to identification of new therapeutic targets and stem cell therapies for wound healing.

P15, The *in vitro* effect of syndecan-3 gene knockout on bone marrow derived mesenchymal stem cells' properties

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Introduction
Inflammation is a central feature of rheumatoid arthritis that affects around 1% of the population and can result in disability and morbidity. The immunomodulatory effects of bone marrow derived mesenchymal stem cells (MSCs) has been widely studied and the recent observations that syndecan-3 (SDC3) is selectively pro-inflammatory in the joint led us to hypothesise that SDC3 might play an important role in MSCs biology.

Materials and Methods
MSCs were isolated from bone marrow of C57Bl/6 WT (n=6) and *Sdc3*$^{-/-}$ (n=6) mice and used to assess the following: flow cytometry (immunophenotype, size and complexity analysis); population doubling time; colony-forming units; osteogenic, adipogenic and chondrogenic differentiation; adhesion properties to type II collagen, fibronectin and laminin and migration properties. Western blotting was used to investigate which signalling pathways are affected by SDC3 loss in mMSCs.

Results
Immunophenotypic analysis indicated similar surface marker expression pattern for both WT and *Sdc3*$^{-/-}$ mMSCs. While both cell types show similar FSC values, the cell complexity in WT mMSCs showed significantly higher values than the *Sdc3*$^{-/-}$. The spread cell surface area of *Sdc3*$^{-/-}$ mMSCs was dramatically lower as compared to WT. The differentiation potential was similar for both WT and *Sdc3*$^{-/-}$ mMSCs. Collagen and fibronectin significantly improved the adhesion of *Sdc3*$^{-/-}$, but not of WT, mMSCs. Also, collagen significantly increases the number of *Sdc3*$^{-/-}$ mMSCs when compared with WT cells. Laminin proved to have no effect. The wound healing assay showed no significant difference between the two cell types. More WT MSCs migrated towards serum or pleiotrophin (PTN) in the transwell assay. Interestingly, *Sdc3*$^{-/-}$ mMSCs cultured on collagen showed a dramatic increase in AKT phosphorylation accompanied by a decrease in ERK1/2 phosphorylation compared with WT controls.

Discussion
The significantly reduced complexity expressed by the *Sdc3*$^{-/-}$ mMSCs might be due to the fact that the cytoplasmic domains of syndecans are commonly involved in cytoskeletal regulation, thus the ablation of *Sdc3*$^{-/-}$ may trigger cellular morphological changes. These morphological changes were confirmed by the significant reduced cell surface spread of *Sdc3*$^{-/-}$ mMSC and appeared to be driven by hyperactivation of the PI3K/AKT pathway at the expense of the ERK1/2 pathway.

P16, Modelling Alport syndrome using patient-derived kidney organoids

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Introduction
Alport syndrome is a rare renal disease caused by mutations in the genes COL4A3, COL4A5 or COL4A5 leading to reduced or absent type IV collagen α3α4α5 networks in glomerular basement membranes (GBM), which underlie all epithelial sheets providing a scaffold for cells.
Although the genetic basis for Alport syndrome has been known for decades, there is incomplete understanding about pathogenic mechanisms. Human pluripotent stem cells (hPSCs)-derived kidney organoids have great potential for understanding kidney development and facilitating disease modelling, and ultimately as a source for renal replacement.

Materials and Methods
Induced pluripotent stem cells (iPSCs) derived from patients with Alport syndrome were obtained from the Human Induced Pluripotent Stem Cell Initiative (HipSci), along with healthy control lines. Both Alport iPSCs and control hPSCs were differentiated to kidney organoids following a modified protocol from Takasato et al. in 2015.

Results
Whole mount immunofluorescence and immunohistofluorescence showed the organoids contain the most of the cell populations in a mature kidney such as podocytes ($WT1^+$, $NPHS1^+$), proximal tubule (LTL^+), distal tubule ($ECAD^+$), and endothelial cells ($CD31^+$), and form an appropriately segmenting nephron structures after 7 days 2D culture followed by 18 days 3D culture *in vitro*. Notably, mature GBM proteins laminin and type IV collagen network were expressed surrounding the glomeruli and between nephrons, which were only observed from animal models or *in vivo* transplanted organoids. Real-time PCR data also suggested the present of laminin and type IV collagen networks in GBM by verifying the gene expression level of LAMB1, LAMB2, and COL4A1, COL4A3, COL4A4, COL4A5, and COL4A6. Primitive foot processes was observed by transmission electron microscopy.

Discussion
Although the *in vitro* grown organoids is not fully developed regarding structure and mature function, we have demonstrated that the organoids generated contain the key components of GBM, therefore would be a promising model for investigation of basement membrane assembly, improvement of mechanistic understanding and would lead to the identification of new therapeutic targets.

P17, A polycaprolactone/glycosaminoglycan scaffold for peripheral nerve regeneration

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Introduction
Nerve fibers of the Peripheral Nervous System (PNS) have a remarkable ability to regenerate and can lead to an almost complete recovery of normal function. This process is governed by glial cells, known as Schwann cells, through their unique capacity to dedifferentiate into cells that drive the healing process. However, post-traumatic nerve repair continues to be a major challenge in restorative medicine and micro-surgery and several studies have been conducted to evaluate the efficiency of acellular nerve grafts. The major challenge in tissue engineering is to develop a synthetic support structure, or scaffold, able to mimic the natural extracellular matrix (ECM). In this respect, it is known that glycosaminoglycans (GAGs) of the extracellular matrix are involved in proliferation, synaptogenesis, neural plasticity as well as regeneration of the PNS. Here, we developed fibrous scaffolds functionalized with GAGs that allowed us to assess their influence on the adhesion, proliferation, and differentiation of Schwann cells.

Materials and Methods
We set up a method to functionalize electrospun scaffolds of polycaprolactone (PCL) fabricated with both random and aligned fibres with GAGs purified from porcine vascular tissue. Neuronal Schwann cells RT4-D6P2T were seeded on scaffolds with or without GAGs.
Proliferation, metabolic activity, and GAGs assays were performed. The expression of specific markers, i.e. Syndecan 1, Syndecan 4, Integrin, Laminin and p75, during 7 days of culture, was evaluated by both immunofluorescence and western blot analyses.

Results
We found that functionalization with GAGs of both aligned and random fiber scaffolds resulted in increased cell proliferation at day 1. In addition, functionalized aligned scaffolds displayed an increase in GAG levels at day 1, probably due to cell extracellular matrix formation, and an increase in syndecan-4 expression at day 7.

Discussion
Overall, cellular colonization studies suggest that PCL–GAG scaffolds could represent a promising artificial substrate that closely mimics the recently established pattern of Schwann cells migration into the regenerating nerve.

P18, Modelling the Brain Tumour Microenvironment

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Introduction
Current *in vitro* models used to study children's brain cancer using 2D monolayer cultures or 3D models containing animal-derived products typically fail to recapitulate the complexity of the brain tumour microenvironment, unreliably represent human extracellular matrices (ECM), and/or raise ethical questions of using animal-derived products in research. Therefore, we aim to optimise a synthetic peptide hydrogel model which can be selectively functionalised with various ECM components to mimic specific tumour microenvironments.

Materials and Methods
The self-assembling FEFEFKFK gelator octapeptide has been used to encapsulate a variety of cell types with excellent viability. Functionalisation of the gel can be achieved by stirring in soluble ECM components during cell encapsulation. We will also apply various additional methods to functionalise the peptide, including direct extension to contain ECM-protein cell-binding motifs and incorporation of non-natural amino acids to enable subsequent addition of functional groups. The effect of these modifications on encapsulated cells will be evaluated using a variety of techniques including fluorescent-confocal microscopy and RT-PCR.

Results
Representative cells have been encapsulated and cultured, including the medulloblastoma cell lines ONS76 and CHLA-01R-MED. We have also established in-gel culture of mouse embryonic stem cell lines E14 and $Ext1^{-/-}$. Differentiation of $Ext1^{-/-}$ cells, lacking endogenous heparan sulphate (HS), has been observed only in gels functionalised with HS, demonstrating the immobilised glycosaminoglycan is biologically active.

Discussion
Initial results demonstrate relevant encapsulated cell types can be cultured and that functionalisation of the gel is associated with characteristic changes in behaviour of at least one cell type. From this, we have constructed a test environment to evaluate the biological effect of modifications to the hydrogel. Our aim is to develop a model that recapitulates the brain tumour microenvironment in a superior way compared to current models, without the need for animal-derived products, for application in the evaluation of new drug treatments to combat children's brain cancer.

Workshop 2: Fibrillar/Matricellular Signalling

P19, ST: The interaction of thrombospondin-1 and -2 with FGF2 in the control of tumor angiogenesis

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Introduction

Thrombospondin (TSP)-1 and TSP-2 are major endogenous inhibitors of angiogenesis. They act through different mechanisms, including interaction with endothelial cell receptors and direct binding to angiogenesis regulatory factors. We previously demonstrated that the type III repeat (T3R) domain of TSP-1 inhibits angiogenesis by binding and sequestering the angiogenic factor FGF2. Since TSP-2 shares with TSP-1 similar structures and functional properties, we hypothesized that also TSP-2 might bind FGF2, ad that the T3R domain could be used as a model for the development of new inhibitors of pathological angiogenesis.

Materials and Methods

The FGF2 binding properties of TSP-1 and TSP-2, recombinant domains, and synthetic peptides were investigated by solid phase binding and surface plasmon resonance assays. Computational docking analysis was used to characterize the binding interface. Non-peptidic, TSP-based compounds identified by pharmacophore-based screening of libraries of small molecules were analyzed for ability to bind FGF2 and inhibit its activity in biological assays.

Results

The T3R domain of TSP-2 bound FGF2, with high affinity (*Kd* in the low nanomolar range) and binding properties similar to the T3R domain of TSP-1. Binding was affected by calcium and heparin. The minimal FGF2 interacting sequence was localized in a 7mer peptide of TSP-1 and the corresponding sequence in TSP-2. The interaction of TSP-2 and TSP-1 with FGF2 showed comparable molecular requirements and resulted in the inhibition of FGF2 binding to both heparin (used as a structural analogue of heparan sulfate proteoglycans) and FGFR-1. Small molecules mimetic of TSP-1 prevented the binding of both TSP-1 and TSP-2 to FGF2, confirming the shared recognition determinants, and inhibited FGF2 interaction with endothelial cell receptors and angiogenic activity.

Discussion

This study identifies TSP-2 as a new FGF2 ligand that shares with TSP-1 similar binding properties and molecular requirements and a comparable capacity to block FGF2 interaction with pro-angiogenic receptors. This likely contributes to TSP-2 antiangiogenic and antineoplastic activity, providing the rationale for future therapeutic applications.

P20, ST: The Structure and Regulation of latent TGFβ by LTBP1 and Fibrillin

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Background
TGFβ plays crucial roles in embryonic development, tissue homeostasis and the pathogenesis of a wide-range of diseases, and the bioavailability of TGFβ is regulated in the matrix by latent TGF-binding protein (LTBP)-1 and fibrillin. Fibrillin binds to LTBP-1 which is covalently linked to proTGFβ, mutations in fibrillin lead to Marfan Syndrome and Weill Marchesani syndrome (WMS) with dysregulated TGFβ signaling indicating that sequestration of TGFβ by fibrillin is essential.

Materials and Methods
Surface Plasmon Resonance binding assays were used to determine kinetic parameters. Transglutaminase cross-linking was analysed by western blotting. TGFβ signaling assays were analysed by blotting for phosphoSmad. Cryo-EM with single particle analysis was used to determine the structure of the ternary complex.

Results and Discussion
We show that the interaction between LTBP1 and fibrillin is strengthened by the presence of the fibrillin N-terminal domain and a WMS-causing deletion in fibrillin reduced binding. Furthermore, a novel LTBP1-fibrillin cross-link by tissue transglutaminase was identified which formed in the presence of covalently-bound proTGFβ. However, head-to-tail assembly of LTBP1 precluded cross-link formation with fibrillin and similarly interaction of LTBP1 with fibrillin prevented LTBP1 head-to-tail assembly. Unexpectedly, the addition of fibrillin to the LTBP1-proTGFβ complex increased TGFβ signaling, indicating that unassembled fibrillin enhances presentation of the complex to the cell surface. This was confirmed by addition of heparan sulphate, which ameliorated this effect, suggesting that matrix incorporation of proTGFβ by fibrillin is required for its sequestration. The cryo-EM structure of the proTGFβ-LTBP1-fibrillin ternary complex showed proTGFβ in its characteristic ring-shaped from which LTBP1 and fibrillin protrude each extending radially from the complex for presentation of specific binding regions.

Our data provide insights into the structure of the proTGFβ-LTBP1 complex and its stabilisation by fibrillin. Cross-linking by tissue transglutaminase may further stabilize the latent complex and control bioavailability. However, these interactions are altered by a WMS-causing mutation. These data indicate that formation of LTBP1 multimers and interaction of LTBP1 with fibrillin are mutually exclusive assembly pathways for LTBP1. Matrix regulation of TGFβ signaling requires incorporation of the latent complex which is perturbed in diseases such as Marfan syndrome and WMS when aberrant matrices are formed.

P21, Cartilage intermediate layer protein 1 (CILP1): A novel mediator of cardiac extracellular matrix remodelling

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Introduction
Heart disease is frequently accompanied by cardiac extracellular matrix (ECM) remodelling, often leading to cardiac fibrosis. In the present study we explored the significance of cartilage intermediate layer protein 1 (CILP1) as a novel mediator of cardiac ECM remodelling.

Materials and Methods
Whole genome transcriptional analyses on human myocardial samples from patients with aortic valve stenosis were performed to determine whether CILP1 expression was associated with cardiac fibrosis. CILP-1 protein levels were determined in human cardiac samples using Western blot. Cardiac CILP1 mRNA levels were assessed by qPCR in mouse models of myocardial infarction and hypertension. The cellular source of myocardial CILP1 expression, its regulation and effect were studied in isolated human and rat cardiac cells.

Results
Transcriptional analysis of human cardiac tissue samples revealed a strong association of CILP1 with many structural (e.g. COL1A2 r^2=0.83) and non-structural (e.g. TGFB3 r^2=0.75) ECM proteins. Gene enrichment analysis further underscored the involvement of CILP1 in human cardiac ECM remodelling and TGFβ signalling. Myocardial CILP-1 protein levels were significantly elevated in human infarct tissue and in aortic valve stenosis patients. CILP1 mRNA levels markedly increased in mouse heart after myocardial infarction, transverse aortic constriction, and angiotensin II treatment. Cardiac fibroblasts were found to be the primary source of cardiac CILP1 expression. Recombinant CILP1 inhibited TGFβ-induced αSMA gene and protein expression in cardiac fibroblasts. In addition, CILP1 overexpression in HEK293 cells strongly (5-fold $p<0.05$) inhibited TGFβ signalling activity.

Discussion
In conclusion, our study identifies CILP1 as a new cardiac matricellular protein interfering with pro-fibrotic TGFβ signaling, and as a novel sensitive marker for cardiac fibrosis.

P22, Internal cleavage and synergy with twisted gastrulation enhance BMP inhibition by BMPER

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Introduction
Bone morphogenetic proteins (BMPs) are essential signalling molecules involved in developmental and pathological processes and are regulated in the matrix by secreted glycoproteins. One such regulator is BMP-binding endothelial cell precursor-derived regulator (BMPER) which can both inhibit and enhance BMP signalling in a context and concentration-dependent manner. BMPER undergoes auto-catalytic cleavage which may be important for BMP regulation, and mutation of this cleavage site causes the skeletal disease Diaphanospondylodysostosis. Twisted gastrulation (Tsg) can also promote or ablate BMP activity but it is unclear whether Tsg and BMPER directly interact and thereby exert a synergistic function on BMP signalling.

Materials and Methods
Human BMPER was expressed in HEK293EBNA cells for small-angle X-ray scattering and electron microscopy structural studies and binding studies with Tsg were performed using surface plasmon resonance. BMP inhibition assays were performed in C2C12 cells with a BMP-responsive luciferase reporter.

Results and Discussion
Human BMPER binds to Tsg through the N-terminal BMP-binding region and this region alone more potently inhibits BMP-4 signalling than full-length BMPER. Additionally, BMPER and Tsg cooperatively inhibit BMP-4 signalling suggesting a synergistic function to dampen BMP activity. Furthermore, full-length BMPER is targeted to the plasma membrane via binding of its C-terminal region to cell surface heparan sulphate proteoglycans but the bioactive cleavage fragment is diffusible. Structural analysis shows that BMPER has an elongated conformation allowing the N-terminal BMP-binding and C-terminal cell-interactive regions to be spatially separated.
To gain insight the regulation of BMPER bioavailability by processing, a disease-causing BMPER point mutation, P370L, previously identified in the acid-catalysed internal cleavage site, was introduced. The mutant protein was secreted but the mutation prevented intracellular cleavage. The full-length mutant protein inhibited BMP-2 as effectively as the wildtype protein but the lack of N-terminal bioactive cleavage product gives an overall reduction in BMP inhibition. Furthermore, the mutant protein was extracellularly cleaved at a downstream site presumably becoming available due to the mutation. This susceptibility to extracellular proteases may result in degradation of the mutant protein coupled with the absence of the bioactive cleavage product results in loss of BMPER function in disease.

P23, High-affinity collagen binding and signalling requires clustering of DDR1 in the cell membrane

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Introduction
The collagen receptor discoidin domain receptor 1 (DDR1) is a receptor tyrosine kinase with key functions in cell regulation, including adhesion, migration and proliferation. DDR1 is considered an attractive therapeutic target for a number human diseases but little is understood about how collagen binding results in intracellular kinase activation. In contrast to classical receptor tyrosine kinases, whose kinase activity is switched on within seconds of ligand binding, DDR1 activation is slow, with autophosphorylation occurring within hours of collagen binding. Our previous studies defined the interaction of the extracellular discoidin domain with the collagen triple helix at atomic-level detail, but how this translates to collagen binding to DDR1 on the cell surface has not been explored.

Materials and Methods
DDR1 distribution and collagen binding on the cell surface was visualised with immunofluorescence. Flow cytometry was used to measure collagen and collagen-mimetic peptide binding to DDR1. DDR1 autophosphorylation was visualised with Western blotting.

Results
Here we show that collagen binding results in clustering of DDR1 in the cell surface with kinetics that are much faster than kinase activation. DDR1 clustering is induced by stimulation with different collagen types as well as collagen-mimetic peptides. DDR1 mutations that block signalling but are far away from the ligand-binding pocket block clustering and collagen binding but do not affect binding of collagen-mimetic peptides. Blocking antibodies that modulate signalling allosterically inhibit collagen-induced clustering and collagen binding to cells.
Additionally, we show that at longer stimulation times, clustered DDR1 redistributes into a more aggregated state which correlates with autophosphorylation.

Discussion
We conclude that high-affinity collagen binding requires DDR1 clustering in the cell membrane, which is dependent on discoidin domain residues far away from the collagen-binding pocket, as well as intact transmembrane helix interactions. Thus, receptor-receptor interactions are required for high-affinity collagen binding to DDR1. Autophosphorylation results from a second-stage, slow DDR1 redistribution into a more aggregated state, indicating kinase activity requires high molecular density.

P24, Targeting organ-specific metastasis through analysis of cancer cell responses to ECM

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Introduction
Metastatic disease accounts for approximately 90% of cancer-related deaths, and understanding this process is of significant therapeutic interest. The tumour microenvironment, composed of stromal and cancer cells surrounded by extracellular matrix (ECM), plays a crucial role in the metastatic process. The ECM scaffold that a cancer cell encounters when it leaves the primary tumour is different in composition depending on the organ to which it travels, and will therefore induce different intracellular signalling when compared to the primary tumour site. In addition, priming from a primary tumour through secreted factors can provide a more favourable environment at the metastatic organ by changing the ECM at that site. Here, we aim to understand the role of metastatic organ ECM on cancer cell signalling to elucidate how these cancer cell-ECM interactions could be targeted to reduce metastatic growth.

Materials and Methods
We have used the 4T1 breast cancer model along with fibroblasts from primary (mammary) and metastatic (lung) sites for *in vitro* investigations. Lung or mammary fibroblasts were cultured to allow the deposition of ECM. Fibroblasts were removed and 4T1 cells were seeded onto the ECM.

Results
Mass spectrometry analysis of the composition of lung and mammary fibroblast-derived ECM correlated well with analysis of decellularised mouse lung and mammary glands, and identified proteins that were higher in lung as compared to the mammary. Lung fibroblasts were also treated with tumour conditioned media from 4T1 cells during ECM deposition to mimic the cancer priming effect. Kinase profiling identified several kinases with higher signalling in 4T1 cells on lung fibroblast-derived ECM when treated with conditioned media. Inhibitors against these kinases also had an effect on the proliferation of 4T1 cells in lung ECM, as seen in a drug screen using a library of 250 kinase inhibitors. Selected kinase inhibitors are now being tested *in vivo* for efficacy against lung metastases.

Discussion
Ongoing studies include analysis of cellular response to liver fibroblast-derived ECM to study a second metastatic organ, and validation of findings in a second cancer type. Our aim is to use our approach to identify how to target organ-specific metastases for individual cancer patients.

P25, ST: Insights into the structure and dynamics of lysyl oxidase propeptide, a flexible protein with numerous partners

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Introduction

Lysyl oxidase (LOX) catalyzes the oxidative deamination of lysine and hydroxylysine residues in collagens and elastin. It is secreted as a proenzyme, which is cleaved by bone morphogenetic protein-1 into the LOX catalytic domain and the N-terminal propeptide (LOX-PP). LOX-PP has anti-tumoral and pro-adipogenic activities.

Materials and Methods

The recombinant human LOX-PP was characterized by circular dichroism (CD), dynamic light scattering (DLS), and small-angle X-ray scattering (SAXS). Five models of the propeptide were built by coarse-grained molecular dynamics simulations restrained by SAXS data. Binding partners of LOX-PP were identified by Surface Plasmon Resonance and Bio-Layer interferometry.

Results

LOX-PP is an elongated protein (Dmax: 11.7 nm) fitting to an envelope of 14.3 nm $\times$ 6.3 nm $\times$ 5.2 nm. The ratio of its radius of gyration, determined by SAXS (3.7 nm), to its hydrodynamic radius, calculated by DLS (3 nm), is higher than 0.8, which indicates that LOX-PP is extended. This is consistent with the presence of intrinsic disorder (64.5% evaluated by CD) and the existence of several conformations based on Ensemble modelling. The N-terminus of LOX-PP appears to be folded in 4 out of the 5 models. LOX-PP is indeed able to fold into a-helix in the presence of trifluoroethanol, and the binding of an hexasaccharide of heparin is predicted to induce partial folding of the propeptide. Heparin-binding sites appeared to be mostly localized at the N-terminus. We have identified 19 new binding partners of the propeptide, including fibrillar collagens, glycosaminoglycans, cross-linking and proteolytic enzymes, one proteoglycan, one growth factor and one membrane protein.

Discussion

Our data suggests new roles for the propeptide in ECM assembly and cross-linking, cell-matrix adhesion, and in the regulation of signaling pathways.

P26, The role of a novel chaperone, CRELD2, in skeletal development and bone homeostasis

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Introduction
Creld2 is expressed in mouse embryonic skeletal tissues and has previously been identified as a genotype-specific endoplasmic reticulum (ER) stress inducible gene implicated in the pathogenesis of skeletal dysplasias caused by mutations in matrilin-3 and collagen X. However, the function of *Creld2* in skeletal development and disease is largely unknown despite putative roles described in protein folding and trafficking.

Materials and Methods
Conditional *Creld2* knockout mice were generated by deleting exons 3-5 in *Creld2* using cartilage (*Col2-Cre*) or bone (*OC-Cre*)-specific *Cre* recombinase. Cartilage and bone-specific *Creld2* knockout mice were deep phenotyped using a variety of morphological and histological techniques. Genetic and signaling changes following the ablation of *Creld2* were analysed by transcriptomic and proteomic methods in order to gain a deeper understanding of its role in bone formation and growth. The results outlined a role for CRELD2 in chondrocyte and osteoblast differentiation, which was confirmed *in vitro* using mesenchymal stem cells (MSCs) and siRNA.

Results
Cartilage-specific *Creld2* knockout mice display a distinctive chondrodysplasia phenotype characterized by disproportionate short statue and a disrupted cartilage growth plate. The ablation of *Creld2* in the growth plate significantly reduces chondrocyte proliferation and survival resulting in regions of hypocellularity and a striking misalignment of chondrocytes within individual chondrons and columns. Interestingly, the primary cilia are also reduced in length, which could impair the columnar organization of growth plate chondrocytes. Bone-specific *Creld2* knockout mice display an osteopenic phenotype characterized by a reduction in bone mass. The deletion of *Creld2* in osteoblasts significantly impairs osteoblast survival and maturation and interestingly induces the up-regulation of osteoblast-derived osteoclastogenic cytokine production, disrupting the balance between bone formation and resorption. The role of CRELD2 in promoting chondrocyte and osteoblast differentiation and maturation was further verified in an *in vitro* system using MSCs and siRNA. We propose that CRELD2 folds and traffics a transmembrane cell-surface receptor that signals to promote differentiation and maturation.

Discussion
In summary, *Creld2* plays distinct roles in bone and cartilage and points to an important role for *Creld2* in controlling chondrocyte differentiation, osteoblast maturation and skeletal development potentially via the modulation of WNT signalling.

P27, ST: Recycled secreted metalloproteases are required for formation of the primary cilium and hedgehog signalling

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Introduction

The primary cilium, an organelle present in most cells, has an indispensable role in cell signalling. Here, the secreted, homologous metalloproteases ADAMTS9 and ADAMTS20 are identified as novel regulators of ciliogenesis and developmental hedgehog signalling.

Materials and Methods

The study utilized combinatorial mouse ADAMTS9 alleles and combined mutagenesis of ADAMTS9 and ADAMTS20, RNA in situ hybridization, immunofluorescence of extracellular matrix and cellular organelles, super-resolution microscopy, scanning and transmission electron microscopy, yeast-two-hybrid cloning, CRISPR-Cas9 inactivation of ADAMTS9 in RPE-1 cells, LRP and clathrin siRNA and rescue by recombinant ADAMTS proteases.

Results

Combined ADAMTS9 and ADAMTS20 mutagenesis in mice led to an open neural tube with defective hedgehog signalling, which is transduced by primary cilia. In addition to extracellular matrix (CSPG, HSPG and fibronectin) accumulation in the neural tube, consistent with the cognate proteolytic activity of ADAMTS9 and ADAMTS20 against proteoglycans, mutant neural epithelial cells had short primary cilia.

Super-resolution microscopy of several cell types identified ADAMTS9 and ADAMTS20 in Rab11+ vesicles encircling the base of cilium. Trafficking analysis showed that vesicular ADAMTS9 was derived from secreted and furin-processed ADAMTS9 initially bound to the cell-surface and subsequently internalized by LRP1 and clathrin-mediated endocytosis. Ciliogenesis was impaired by CRISPR-Cas9 inactivation of *ADAMTS9* in RPE-1 cells and restored by either transfected or exogenous ADAMTS9 and ADAMTS20, which trafficked to the cilium base. Catalytically active ADAMTS9 or ADAMTS20 but not their proteolytically inactive mutants nor related matrix-degrading proteases ADAMTS1 and ADAMTS5 could rescue the defect in mutant RPE-1 cells. Imaging of sequential steps in ciliogenesis using super-resolution microscopy and electron microscopy suggested that endocytosed ADAMTS9 and ADAMTS20 mediate ciliary vesicle expansion and uncapping of the mother centrosome, which are prerequisites for ciliary axoneme extension. Analysis of pericellular proteoglycans in RPE-1 cells suggested an uncoupling of ECM proteolysis from ciliogenesis.

Discussion

The findings show that in addition to a canonical role in matrix proteolysis, ADAMTS9 and ADAMTS20 have an unexpected and independent role in formation of the primary cilium. Their dual function is ensured by initial secretion and cell-surface binding for pericellular ECM proteolysis and subsequent internalization to a unique pericentriolar vesicle population mediating ciliogenesis.

P28, Biglycan triggers the recruitment of Th1 and Th17 cells into the kidney through the TLR2/4/MyD88/TRIF-signaling

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Introduction
Biglycan is a proteoglycan which is sequestered in the extracellular matrix under physiological conditions. Upon injury, soluble biglycan is released and signals in macrophages via different signaling pathways. Thus, biglycan engages TLR2 and/or TLR4 receptors and their adapters, MyD88 and/or TRIF, and mediates the inflammatory response.
T helper (Th) 1 and Th17 cells are important factors in the progression of chronic kidney diseases (CKDs). While CXCR3+ Th1 and Th17 cell chemoattraction is mediated by CXCL9 and CXCL10, the CCR6+ Th17 cell recruitment is dependent on CCL20.
Aims: 1) to study the role of biglycan in the Th1/Th17 cell recruitment in murine streptozotocin (STZ)-induced diabetes; 2) to elucidate the mechanism of biglycan-mediated Th1/Th17 recruitment in macrophages.

Methods
In vitro: Mouse peritoneal macrophages (WT, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, *Tlr2*$^{-/-}$*/Tlr4-m, Myd88*$^{-/-}$*, Trif-m, Myd88*$^{-/-}$*/Trif*$^{-/-}$) stimulated with biglycan. *In vivo*: WT, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, *Tlr2*$^{-/-}$*/Tlr4-m, Myd88*$^{-/-}$*, Trif-m, Myd88*$^{-/-}$*/Trif*$^{-/-}$ mice transiently overexpressed with biglycan. STZ-induced diabetes in biglycan-deficient or transiently overexpressed mice. *Techniques:* flow cytometry, immunohistochemistry, qPCR, Western blot, ELISA.

Results
Biglycan deficiency ameliorates the renal influx of Th1/Th17 cells and the subsequent production of IFNγ/IL-17 in STZ-induced diabetic mice. On the contrary, the transient overexpression of biglycan in STZ-treated mice results in more pronounced renal infiltration of Th1/Th17 cells and generation of IFNγ/IL-17. Moreover, the production of CXCR3+ Th1/Th17 cell chemoattractants, CXCL9/CXCL10 and CCR6+ Th17 cell chemoattractant, CCL20 is dependent on biglycan in diabetes. In macrophages, biglycan induces the Th1/Th17 cell chemoattractants through three different mechanisms. Biglycan induces the CXCL9/CXCL10 production through TLR4/TRIF, while CCL20 via TLR2/4/Myd88. Remarkably, biglycan induces CXCL9 in acrophages only in presence of IFNγ via TLR4/TRIF.

Discussion
Biglycan is a novel trigger of Th1/Th17 cell recruitment into the kidney and we propose the biglycan/TLR/TRIF/MyD88-signaling axis as a therapeutic target in CKDs.

P29, Unusual collagens in the *C. elegans* matrisome – a new classification of cuticular collagens

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Introduction
Collagens form the most abundant protein family in the human body and are important in development, homeostasis and disease. Recently, we demonstrated that they are also important for longevity in *C. elegans* (Ewald et al. Nature 2015). In analogy to the efforts to characterise all extracellular matrix (ECM) proteins as well as ECM-associated proteins in humans (termed “matrisome” (Naba et al. MCP 2012)), we used bioinformatic methods to defined the matrisome in *C. elegans*. Here, we focus on the characterisation of collagens and their unusual organisation in nematodes.

Materials and Methods
We identified collagens by a combination of bioinformatics analysis and manual curation. Classification were performed by manual pattern recognition assisted by bioinformatics.

Results
With 184 members, the category of collagens is the largest category in the core matrisome of *C. elegans*. Interestingly, true homologues are only present for collagen IV. Six other collagens have similarities in terms of their domain arrangements but do not share any significant sequence similarity. Strikingly, classical fibrillar collagens (like collagen I or II) are completely absent in *C. elegans*.
172 collagens belong to the group of cuticular collagens, defined by a relatively short collagenous domain (approx. 40 GXY triplets), which is flanked by an N- and C-terminal cysteine knot. Similar to fibrillar collagens, there is an additional N-ProHelix of usually 10 GXY repeats stabilised by another cysteine knot. We further clustered these collagens into 4 main groups, subdivided into 74 clusters – based on the idea that protein within one cluster should be more likely to form heterotrimers. Many (117) cuticular collagens are predicted to be transmembrane; however, most of them carry a furin cleavage site, indicating shedding. Interestingly, there is no obvious C-Propeptide suggesting other mechanisms of chain selectivity and probably even triple helix formation.

Discussion
Our proposed classification helps understand observed phenotypes as well as identifying promising candidate genes for future investigations. We already started initial experiments to determine the spatio-temporal localisation of collagens by GFP-fusion constructs, as well as analysis of the biochemical characteristics of these unusual collagens. Together with the newly defined matrisome, this classification will help interpret future findings in genetic screens and proteomic analyses.

P30, Differential dynamics of fibrillar collagen-binding integrins in cell-collagen interactions

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Introduction
Integrin α2β1 and α11β1 are two collagen receptors which in addition to sharing sequence similarity also share similar preference for fibrillar collagens, suggesting that these integrins could have similar functions when co-expressed in mesenchymal cells. Available data however suggest that these collagen-binding integrins have different functions both *in vitro* and *in vivo*. Although both integrins are expressed on fibroblasts, only α11β1 has been reported to contribute to myofibroblasts differentiation and to regulate cardiac and wound fibrosis. How these two integrins transduce distinct functions is unclear.

Methods
We investigated the potential differences between the two fibrillar collagen-binding integrins, α2β1 and α11β1, with a special focus to their distribution in cell-matrix adhesions using primary human gingival fibroblasts and C2C12 cells expressing chimeric constructs, where the cytoplasmic tail of integrin α2 was swapped with α11 cytoplasmic tail (α2Xα11C-mcherry) or vice-versa (α11Xα2C-EGFP).

Results
Integrins α2β1 and α11β1 displayed different cell surface dynamics during matrix remodeling and assembly in human gingival fibroblasts. Whereas α2β1 integrin in spreading fibroblasts remained localized to focal adhesions at the periphery of the cells, α11β1 appeared to slide from focal adhesions into central adhesions. Integrin α11β1 in central adhesions co-localized both with its ligand, fibrillar collagen type I, and the fibrillar adhesion markers tensin-1 and Kank2, thus establishing α11β1 integrin as a novel marker for fibrillar adhesions. To better understand the molecular mechanism explaining why α11β1, but not α2β1, translocates into fibrillar adhesions, we exchanged the cytoplasmic tail of integrin α2 with that of α11, and vice versa. Replacing integrin α11 cytoplasmic tail with the cytoplasmic tail of α2 (α11Xα2C-EGFP) strongly reduced α11β1 fibrillar adhesions, whereas replacing α2 cytoplasmic tail with the α11 cytoplasmic tail (α2Xα11C-mcherry) did not result in integrin α2β1 becoming localized in fibrillar adhesions.

Discussion
Our results strongly suggest that both the extracellular part and cytoplasmic tail of integrin α11 are involved in the α11β1-dependent stabilization of fibrillar adhesions and further studied are needed to identify the molecular mechanisms underlying the differential distribution of α2β1 and α11β1.

P31, Integrin α11 cytoplasmic tail is required for FAK activation to initiate 3D cell invasion and ERK-mediated cell proliferation

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Introduction
Integrin α11β1 is a collagen-binding integrin, which is receiving increasing attention in the context of wound healing, fibrosis and tumour-stroma interactions. Integrin α11β1 has been reported to contribute to myofibroblasts differentiation and could be a potential target to control cardiac fibrosis. The detailed molecular mechanisms through which integrin α11β1 mediates fibroblast cell functions are poorly understood. To better understand the signaling function of integrin α11β1, we addressed this question by characterizing the role of integrin α11 cytoplasmic tail.

Materials and methods
We investigated the role of integrin α11 cytoplasmic tail by generating a truncated variant of α11 with a deletion of the terminal 17 amino acid residues in the α11 tail. C2C12 cells lacking collagen-binding integrins were transfected with wildtype α11 or α11-tail less constructs to study the effect of this deletion in integrin α11 function.

Results
C2C12 cells expressing tail-less α11 attached normally to collagen I, but displayed fewer focal contacts on collagen I. Integrin α11-tail less cells furthermore displayed a reduced capacity to invade and reorganize a 3D collagen gel and proliferated less when compared to wild type cells. Analysis of cell signaling showed that α11-mediated FAK and ERK phosphorylation was reduced in cells expressing tail-less α11. ERK and FAK inhibitors inhibited α11-mediated cell proliferation, whereas α11-mediated cell invasion was FAK-dependent and occurred independently of ERK signaling.

Discussion
Our data demonstrate that the integrin α11 cytoplasmic tail plays a central role in α11 integrin specific functions, including the FAK-dependent ERK activation and suggest that α11-tail can regulate fibroblast adhesion, migration, and proliferation in a fibrillar collagen matrix.

P32, Chondrocyte specific deletion of CCN2 does not exacerbate osteoarthritis in models of post-traumatic osteoarthritis in mice

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Introduction

CCN2 is a matricellular protein expressed in both healthy and osteoarthritic cartilage. It enhances the production of aggrecan and collagen type II, whilst also promoting the proliferation, differentiation, and maturation of growth plate chondrocytes. CCN2 null mice exhibit a range of skeletal dysmorphisms, highlighting the importance of CCN2 in regulating matrix formation and turnover. The aim of this study was to determine the function of CCN2 in chondrocytes in models of trauma-induced osteoarthritis (OA).

Materials and Methods

CCN2 gene deletion was induced specifically in chondrocytes following tamoxifen treatment in male CCN2 floxed mice aged 8 weeks. OA was induced either through surgical injury, or non-invasively by applying a controlled loading programme, to the tibio-femoral joint. Knee joints were harvested, scanned with µCT, and processed for histology. Sections were stained Safranin-O or Toludine Blue, and scored for cartilage degradation using the OARSI grading system.

Results

In the surgical model of OA, cartilage degeneration was more severe in the medial tibia of CCN2 KO mice compared to WT with maximum scores averaging 5.28 (±0.28 SEM) and 4 (±0; p=0.02) for KO and WT respectively, at 4 weeks post-surgery. No significant differences were observed at 8 weeks post-surgery. µCT analysis showed no significant differences in subchondral bone thickness, trabecular bone BV/TV, and trabecular thickness between WT and KO mice at either time point. In the non-surgical model of OA no significant differences in the severity of OA lesions were observed between KO and WT at 6 weeks post-loading. µCT analysis showed no significant differences between KO and WT loaded knees.

Discussion

The preliminary data obtained suggests that CCN2 may play a protective role in limiting cartilage degeneration in the early stages following initiation of OA, however this effect appears to be lost with time. The progression of OA in both models at later time points suggests that CCN2 expression in chondrocytes does not prevent OA, however analysis of earlier time points may reveal further beneficial effects of CCN2 and its role in OA pathogenesis.

P33, Identifying the Binding Sites of the Small ECM Protein Dermatopontin on Fibrillary Collagens

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Introduction
Dermatopontin (DPT) is a 22 kDa acidic collagen-binding protein that enhances collagen fibrillogenesis *in vitro* and is retained on the fibrils after fibrillogenesis. However, the binding sites on collagens are unknown. DPT also interacts with a large range of other ECM proteins, growth factors and cell surface receptors. DPT has been hypothesized to act as an adaptor between cells and ECM due to the interactions with ECM proteins, both fibrous and smaller, and cell surface receptors. DPT plays a role in wound healing and is involved in several fibrotic disorders. Previous studies have shown that knock-out of *Dpt* in mice results in thinner dermis and cornea, abnormal fibrils, changes in fibril organization and a Ehlers-Danlos-syndrome-like phenotype.

Materials and Methods
DPT was extracted from six different collagenous tissues and purified from the cornea. DPT´s binding sites on fibrillary collagen are revealed through the use of the Collagen Toolkits. The Collagen Toolkits are collections of overlapping triple-helical peptides covering the triple-helical domains of collagen II and III. The peptides were arrayed in 96-well plates, together with DPT, in order to map the binding sites.

Results
Extractions from collagenous tissues suggest that the highest amount of DPT is found in the cornea, which was consequently chosen as the source for obtaining purified endogenous DPT for further characterization of the protein. Far-western blotting of tissue extracts, utilizing DPT as the probe protein, reveal an affinity for different types of collagens. Results from using the Collagen Toolkits reveal several binding sites along the triple-helical domains varying in affinity. Further experiments and data treatment indicate that specificity for these sites is based on a combination of ionic and hydrophobic interactions.

Discussion
The discovered binding sites may contribute to understand DPT´s functions *in vivo* and suggest an interplay with other collagen-binding proteins. The identified binding sites could also provide an explanation for why *Dpt* knock-out introduces a Ehlers-Danlos-syndrome-like phenotype in mice and consequently a possible role of DPT in this disorder in humans.

P34, COMP is not a cartilage protein in zebrafish

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Introduction
COMP (Cartilage Oligomeric Matrix Protein) is a member of the thrombospondin family of extracellular matrix proteins and is also referred to as thrombospondin-5 (TSP-5). It has been proposed that COMP has evolved rapidly from a TSP-4-like sequence as an innovation in the tetrapod lineage. Here we present evidence that COMP occurs already in zebrafish and show how we use the zebrafish to study disease mechanism and screen for novel therapeutic approaches for COMP related chondrodysplasias.

Materials and Methods
In silico analysis and phylogenetic analyses were performed to identify zebrafish COMP. The cDNA was cloned, recombinant COMP expressed and affinity purified antibodies generated. In situ hybridization, immunohistochemistry, RT-PCR and immunoblots revealed the temporal and spatial expression and oligomerisation of COMP. Mutant zebrafish strains were generated by CRISPR-Cas.

Results
Zebrafish COMP has a domain structure identical to that of tetrapod COMP and a 73% sequence similarity with murine COMP. The gene is located on chr 11 and has shared synteny with its mammalian orthologs. Recombinantly expressed COMP forms pentamers. COMP is expressed from 2hpf on and COMP protein is first visible in somites at 11 hpf. During development and in adults COMP is expressed in myosepta, craniofacial tendon and ligament, but not in cartilage. In addition, in adults COMP is expressed around ribs and vertebra. Pentameric COMP is easily extractable from 72 hpf whole zebrafish with TBS. Targeting of the first TSP type 3 repeat by CRISPR-Cas resulted in the generation of complete knockouts and three mutant lines with deletions in a calcium binding motif were obtained.

Discussion
The lack of COMP expression in zebrafish cartilage implies that COMP has acquired a new function in tetrapods. The expression in myosepta is reminiscent to that of Drosophila thrombospondin which is required for muscle-specific adhesion to tendon cells. The mutant COMP lines generatedwill be interesting models to study the impact of COMP mutations on secretion and matrix assembly.

Workshop 3: Rhythms and Matrix Dynamics

P35, Honeycomb-like convex structures at the bottom of the dermal layer function as "anchoring structure" to maintain skin elasticity and morphology

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Introduction
The dermal layer is composed of abundant extracellular matrix, and contributes to skin firmness and morphology. These functions require that the dermal layer should be properly anchored to subcutaneous tissue, but little is known about how this is achieved. Here, we aimed to clarify the mechanism involved.

Materials and Methods
90 facial and 70 body (upper arm and abdominal) skin specimens were prepared from surplus skin excised during plastic surgery. Dermal layer structures were observed three-dimensionally (3D) by micro X-ray computed tomography (micro-CT), and their composition was identified immunohistochemically. Skin-retaining force was measured non-invasively with a Cutometer MPA 580® (6 mm probe), and movement of the internal skin structures was observed by ultrasonography. We also examined their relationship to skin morphology, evaluated in terms of sagging (ptosis) severity at the cheek of female volunteers.

Results
Micro-CT observation of the 3D structure of the skin specimens revealed that facial skin contains characteristic convex structures at the bottom of the dermal layer. They were connected to form an overall honeycomb-like appearance. Scanning electron micrography (SEM) showed that the convex structures contain vertically directed collagen fibers to the skin surface, in contrast to the bulk dermal layer, where the collagen fibers are directed horizontally. EVG staining revealed similar orientations of elastic fibers. Thus, the convex structures may serve to retain the dermal layer vertically. Indeed, when we pulled up the skin vertically, we found that the dermal layer is retained tightly on subcutaneous tissues through the convex structures. The retaining force, measured with a cutometer (-Uv/Ue), was significantly positively related to the depth of the convex structures in the cheek of female volunteers. Furthermore, facial sagging severity was significantly negatively related to the depth of these structures. The convex structures significantly decreased with aging, and the decrease was significantly related to the degree of facial sagging.

Discussion
Honeycomb-like convex structures appear to serve as "anchoring structure" to retain the dermal layer, contributing to skin elasticity and superficial morphology. They could be a key to understanding the function of the dermal layer.

P36, *In situ* repair of full-thickness cartilage defects using biomimetic biofunctionalised implants.

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Introduction:
Cells use the heparin interactome as a 'toolkit' for phenotypic change. We hypothesised that taking a biomimetic approach may aid healing of traumatic and early osteoarthritic cartilage.

Materials and Methods:
Poly-L-lactic acid random-fibre scaffolds were electrospun and the surface charge was altered by allyl amine plasma polymerisation followed by charge adsorption of heparin to which pmol amounts of TBFβ3 and CXCL12 were non-covalently bound. The scaffold was assessed *in vitro* for support of long-term cell viability and chondrogenesis by bone-marrow mesenchymal stem cells (MSCs) and primary chondrocytes. Cartilage formation was determined by measuring glycosaminoglycan content. The scaffolds were assessed for *in vivo* efficacy by implantation into surgically created full-thickness chondral lesions in the medial femoral condyles of sheep. Subchondral bone micro-fracture was used to release bone marrow MSCs into the joint. At 4 and 16 weeks, implanted joints were retrieved and cartilage regeneration assessed macroscopically and histologically.

Results:
In vitro, functionalisation of the PLLA scaffold with a combination of TGFβ3 and CXCL12 promoted MSC attachment and ingress throughout the implant, and chondrogenic differentiation. Viability of the cells within the construct was maintained for at least 5-6 weeks in the absence of added serum/growth factors. The MSCs underwent chondrogenesis and produced significantly more ECM than non-functionalised or partially functionalised scaffolds. *In vivo*, implantation of TGFβ3- and CXCL12-functionalised implants, but not empty defects or control, non-functionalised implants, showed regeneration of hyaline neocartilage occurring as early as 4 weeks.

Discussion:
These results demonstrate the potential utility of heparin interactome proteins in the regeneration of diseased or injured tissues. It is feasible to develop off-the-shelf medical devices for the repair of injured or early osteoarthritic cartilage thereby bypassing potential problems associated with cell therapies.

P37, ST: Abnormal fibrillinogenesis leads to disruption of clock gene regulation and reveals an important role for fibrillar matrix in the maintenance of circadian rhythmicity

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Introduction
Abnormal fibrillinogenesis is associated with connective tissue disorders (CTD), including Marfan syndrome (MFS), Systemic sclerosis (SSc) and Tight-skin (TSK). Transforming growth factor β (TGFβ) has been implicated in the CTD pathogenesis. Fibrillin-1 (*Fbn-1*) is known to regulate TGFβ bioavailability and TGFβ, in turn, stimulates *Fbn-1* assembly. Emerging research demonstrated that the molecular circadian clock regulates TGFβ signalling and that TGFβ reinforces circadian rhythmicity through the clock output transcription factors. However, the upstream matrix mediators involved in relaying the temporal TGFβ signal to the clock have not been studied, nor the contribution of circadian clock to the CTD pathogenesis.

Materials and Methods
We have investigated the diurnal expression of core and auxiliary clock components in a genetic mouse model of *Fbn-1* partial duplication (TSK+/-) over a ~24h day in several peripheral tissues using qPCR and immunohistochemistry. Primary adult skin and lung fibroblasts isolated from age-matched wild-type and TSK+/- mice as well as human fibroblasts from SSc and MFS patients were clock synchronised and clock reporter activity monitored using real-time-bioluminescence imaging. Pharmacological clock manipulation was used to assess temporal cell responsiveness to TGFβ. siRNA approach was used to knockdown *Fbn-1* in mouse and human cells and human *Fbn-1* promoter activity was assayed using dual luciferase reporter assays.

Results
We show that mutant *Fbn-1* in TSK+/- mice results in the transcriptional dysregulation of the circadian clock and altered diurnal expression of core and auxillary clock genes. This phenotype is cell-autonomous as it is recapitulated in clock-synchronised primary skin and lung fibroblasts from TSK+/- mice. In-depth analysis of circadian activity using real-time bioluminescence imaging showed altered circadian rhythmicity, including both the amplitude and timing of peak reporter oscillation in TSK+/- fibroblasts as well as human patient fibroblasts. Whilst control fibroblasts show clock-gated responses to TGFβ stimulation, such temporal responses are blunted in fibroblasts with mutant *Fbn-1*. Selective pharmacological clock manipulation blocks TGFβ-induced gene expression, which is dependent on the functional *Fbn-1* matrix. Moreover, *Fbn-1* mRNA shows rhythmic expression in tissues and cells, which is, in part, regulated through the conserved E-box elements in the *Fbn-1* promoter.

Discussion
These results demonstrate bi-directional regulation between fibrillar matrix and the circadian molecular clock and suggest that altered circadian clock as a result of mutant *Fbn-1* may contribute to aberrant temporal regulation of TGFβ signalling in fibrillinopathies.

P38, The effect of [-1A]TIMP3 overexpression on bone mass in the naturally developing OA STR/Ort mice

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Introduction
Osteoarthritis (OA) is the most prevalent degenerative joint disorder that affects a large proportion of ageing population, for which there is no definitive treatment. OA progression affects not only articular cartilage integrity but also bone. For example, osteophyte formation and subchondral bone thickness. It has been previously shown that [-1A]TIMP3 (TIMP-3 with an extra alanine residue at -1 residue) can selectively inhibit ADAMTS-4 and -5, while it is a poor inhibitor of collagenases. The aim of this work is to investigate the *in vivo* effect of [-1A]TIMP3 overexpression on bone tissue in a spontaneously developing OA mouse model (STR/Ort mouse) and evaluate the impact of long term aggrecanase inhibition on bone in OA treatment.

Materials and Methods
We generated several transgenic mouse lines that stably overexpressed [-1A]TIMP3, either conditionally in chondrocytes (using col2a1 promoter) or in all tissues (ELF1-promoter) using lentiviral constructs, in the STR/Ort mouse background. The mice were screened for the [-1A]TIMP3 overexpression by qPCR and bred to establish different levels of TIMP-3. At the age of 40 weeks, mice were sacrificed and hindlimbs were scanned by µCT for bone microarchitecture evaluation and were decalcified and processed for histological analyses of the knee joints. Adult bone cells were also infected with [-1A]TIMP3 to validate the effect.

Results
A total of 34 STR/Ort mice were examined by µCT measuring the tibial trabecular bone parameters of the distal metaphysis and compared with wild-type age matched STR/Ort and mice with B6CBA mixed background. [-1A]TIMP3 mRNA expression in the hips did not differ in the STR/Ort mice in terms of gender and site (left/right). Bone mass was substantially higher in STR/Ort mice as compared with WT, and in STR/Ort females in comparison to males, in both conditional and global overexpression. *In vitro* data show significant increased in mineralization of osteoblasts infected with [-1A]TIMP3 compared with TIMP3 and empty virus.

Discussion
We have previously shown that TIMP-3 overexpression leads to loss of bone mass. the data presented here show that [-1A]TIMP3 which inhibits primarily aggrecanases leads to increased bone mass. This suggests that the balance between aggrecanases and collagenases during bone formation or even adult bone is critical to maintaining healthy bone. It also suggests that [-1A]TIMP3 may potentially be useful in osteoporosis.

P39, The Role of the Circadian Clock Regulated Vacuolar Protein Sorting (VPS) Tethering Complex in Collagen I Turnover and Cell Contractility

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Introduction
Deregulation of extracellular matrix (ECM) forms the basis to many pathological conditions, including fibrosis, tendonopathies, and cancer. Fibroblasts are the major cell type that produces ECM in the body; as such, understanding how ECM production and turnover is controlled in fibroblasts is key to discovering processes that could be targeted for potential therapeutics. Previously we have identified that ECM production and turnover in fibroblasts is regulated by the circadian rhythm. In particular, a member of the vacuolar protein sorting (VPS) family of tethering complexes in protein sorting, VPS33b, is found to be rhythmic from RNA microarray data. VPS33b has been implicated in post-Golgi sorting of protein cargoes, as well as maturation of endosomes/lysosomes. Here, we hypothesise that VPS33b is crucial to the circadian control of both collagen I secretion and uptake.

Materials and Methods
Using CRISPR-Cas9 and shRNA, we have knocked-out VPS33b expression in immortalised Per2-luc mouse tail tendon fibroblasts. Immunofluorescence staining, collagen contraction assay, scratch-wound assay, and lumicycle analyses were carried out. Proteomics studies were also carried out on tail tendon collected from mice at different time points to further investigate the circadian-regulated proteome.

Results
Collagen I has a higher retention rate within the Golgi complex of VPS33b knock-out (VPS33b KO) fibroblasts, and KO cells have a marked reduction in collagen fibril deposition. KO cells also have decreased contractility, and showed decreased amplitude in their circadian rhythm; however, there is no obvious change in the migratory function of VPS33b KO fibroblasts. Proteomics results identified other circadian-regulated VPS family members which may also be involved in extracellular matrix secretion and turnover.

Discussion
VPS33b is crucial for collagen I secretion and deposition of collagen I fibrils. In addition, the effects of VPS33b KO in fibroblast functionality and circadian rhythm suggest that VPS33b may have functions in pathways not related to trafficking, or that VPS33b-regulated protein trafficking is involved in regulating fibroblastic functions. Further analyses on the effects of VPS33b KO on uptake of pre-existing collagen fibrils, as well as the mechanisms of VPS33b-regulated collagen I secretion are currently underway.

P40, Lumican inhibits *in vivo* melanoma metastasis by altering matrix- effectors and invadopodia markers

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Introduction
Lumican, a small leucine-rich proteoglycan, was reported to inhibit the membrane type matrix metalloproteinase MMP-14 activity and melanoma cell migration *in vitro* and *in vivo*. MMP-14 has been implicated in the migratory and metastatic potential of cancer cells. Moreover, Snail was reported to increase EMT and the metastatic potential of cancer cells. Therefore, the aim of this study was to analyse the effect of lumican on Mock and Snail overexpressing B16F1 cells *in vivo*.

Materials and Methods
Intravenous injections of Mock-B16F1 and Snail-B16F1 cells (n= 250x105 per IV) were performed in Lum+/+ (n= 24) and in Lum-/- (n=24) mice. At day 24, mice were sacrificed and lungs were collected. Apart from the in vivo experiments, in vitro methods, like confocal immunofluorescence, real-time PCR, and western blots were conducted, too.

Results
The number of metastatic nodules was significantly higher in mice injected with Snail overexpressing B16F1 cells than in mice injected with Mock-B16F1 cells in both group of mice. In addition, endogenous lumican of wild-type mice significantly inhibited the number of metastatic nodules as compared to lumican deleted mice. Moreover, *in vitro*, lumican inhibited the expression of cortactin (an invadopodia marker), CD44 (hyaluronan receptor), and heparanase. Thus, lumican is able to inhibit the expression of key molecules involved in cancer invasion and metastasis which might explain, at least in part, its inhibitory effect on lung metastatic nodules formation. In addition, the effect of lumican was observed *in vitro* in 3D invasion assays using scanning electron and confocal microscopy. Lumican was able to alter the formation of lamellipodia associated with a more rounded cell shape. Finally, lumican was shown to inhibit the phosphorylation of FAK, Akt, p130Cas and GSK3 α/β.

Discussion
Altogether, the results suggest that a lumican-based strategy targeting Snail-induced metastasis could be a useful therapeutic for melanoma treatment.

P41, Dynamic expression of matrix components during skeletal development

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Introduction
A dynamic and complex collagen network is essential for normal functioning of cartilage and bone. Understanding how this network emerges during development would be critically useful both for understanding the degeneration of skeletal tissues and for tissue engineering of replacement cartilage and bone. However, when and how the key collagens in cartilage and bone develop remains unclear. We examine how the structural organisations of collagen type I, II, III, V, VI and X emerge in the rudiments of the murine forelimb during prenatal development.

Materials and Methods
Mouse embryos were harvested and staged according to Theiler stages TS22, TS25 and TS27 (typically embryonic days 13.5, 15.5 and 17.5). Collagen distribution was studied with immunofluorescence and confocal microscopy.

Results
Initially, collagen I was expressed at the presumptive joint line (TS22 being prior to cavitation), and perichondrium. At TS25 it was expressed in the bone collar and by TS27, it was only observed at the site of endochondral ossification. Collagen II was consistently expressed in cartilage but was absent from mineralised and presumptive mineralised regions. At TS22–TS25, collagen II appeared mesh-like but this patterning was less prominent at TS27. At all stages examined, collagen III was expressed throughout the rudiment including the mineralised cartilage with a pericellular expression. Collagen V was initially expressed throughout the diaphysis but became restricted to mineralised cartilage at TS25–TS27. At all stages examined, collagen VI was detected throughout the rudiment with a pericellular expression pattern. In the growth plate of TS25–TS27 rudiments, it was prominently stacked in rows and had a fibrilar distribution in the mineralised cartilage. At TS22 Collagen X was expressed in the primary ossification centre. Later (TS25–TS27) collagen X distribution was only observed in the growth plates.

Discussion
All but Collagen III had a dynamic expression in the developing skeletal rudiment, offering novel insights into when and how the key collagens in cartilage and bone develop. We propose that these dynamic expressions and compositional arrangements are critical to normal growth, morphogenesis and maturation of developing cartilage and bone, with implications of understanding the disease and regeneration of skeletal tissues.

P42, A transgenic mouse model to visualise the dynamics of a core clock factor BMAL1 in matrix-rich tissues

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Introduction
The circadian clock machinery plays fundamental roles in tissue homeostasis. Molecular clocks in various cell types share a common transcription-translation feedback loop, although it regulates the expression of tissue specific genes in vivo. Our lab has demonstrated a core clock factor BMAL1 is essential for tissue homeostasis and integrity of the musculoskeletal system. In this study, we aim to investigate the dynamics and function of BMAL1 in cells from matrix-rich tissues, such as cartilage and intervertebral disc.

Materials and Methods
We generated a novel mouse model using CRISPR-Cas9 technique, with a Venus reporter knock-in to the Bmal1 gene. Temporal BMAL1 protein abundance and dynamics in tissues and cells were evaluated by live imaging. Also, we measured BMAL1 kinetics using fluorescence recovery after photobleaching (FRAP), as well as BMAL1 concentration using fluorescence correlation spectroscopy (FCS).

Results
The Bmal1::VENUS mice showed similar circadian locomotion activity as wild type mice, indicating BMAL1 functions within the clock were maintained. We found the expression of BMAL1 protein was mainly in the cell nucleus (e.g. chondrocytes and fibroblasts). There is little evidence of 24-hour rhythms for BMAL1 abundance in these cell types, which is consistent with the long half-life (~9 h). Although BMAL1 protein appeared stable, it was highly dynamic with a concentration between 15-25 nM, comparable to the concentration of PER2 proteins.

Discussion
The Bmal1::VENUS mice allow us to study BMAL1 protein in live tissues and cells. Our data suggest that rhythmic oscillation of circadian-controlled genes do not require the levels of BMAL1 to be rhythmic. These results provide new insights to investigate the function of clock machinery in mammalian cells.

P43, ST: Monitoring collagen fibre formation and turnover using CRISPR/Cas9 knock-in of Dendra2

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Introduction

Collagen accounts for 30% of body mass where it functions as a major tissue scaffolding protein and stress-shields cells from destructive mechanical forces. Dysregulation in the amount of collagen is associated with fibrosis (excess collagen) and tissue degenerative (low collagen), for which few therapies and treatments are currently available. Understanding how collagen is produced, processed and regulated is key to developing new treatment strategies. A major technical hurdle has been the absence of a robust method to quantitatively image, in living cells, the production of newly formed collagen, and no high-throughput assay exists.

Materials and Methods

Here we have generated mouse 3T3 cells that have been modified using CRISPR/Cas9 to integrate a Dendra2 fluorescent fusion protein downstream of the signal peptide of the COL1A2 protein. Integration of the reporter maintains the natural context of the genomic structure, ensuring gene expression patterns are maintained. Dendra2 is photoswitchable, which naturally fluoresces green, until exposed to 405nm light which converts the reporter a red fluorescent protein. With this approach we can selectively remove the background collagen signal, and then image or assay specifically for the production of new 'green' collagen.

Results

A first result showed that a bulk of newly formed procollagen traffics through the Golgi apparatus in ~ 3 hours, and this flux is occurs once during 24 hours. This approach allows measurement of the dynamics of procollagen translation and vesicle secretion whilst under endogenous control mechanisms. Dendra2-collagen fibres form following 4 days in culture in the presence of L-ascorbic acid. Fibres are formed within the cell boundary and cells then migrate away from the formed fibrils. Furthermore, individual cells are able to produce multiple fibres. The vast majority (>95%) of collagen fibrils remain stable for up 7 days, however some fibrils are broken down by cells over the course of 24 hours.

Discussion

In conclusion, CRISPR/Cas9 technology has enabled us, for the first time, to live image fibroblasts synthesising collagen fibres at the cell-matrix interface. This technology is likely to be applicable to the study of other fibril-forming collagens or matrix macromolecules.

P44, ST: Distribution and production logistics for *de novo* basement membrane formation

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Introduction

The basement membrane (BM) is a thin layer of extracellular matrix (ECM) beneath nearly all epithelial cell-types that is critical for cellular and tissue function. It is composed of numerous components conserved among all bilaterians; however, it is unknown how a fully mature BM is constructed in the living animal, with all the components prepared at the appropriate amount, time, and place.

Materials and Methods

To address these questions, here we exploit our ability to live image and genetically dissect *de novo* BM formation during *Drosophila* development, which allows us to examine production and distribution of core BM components from their initial induction in the embryo. Taking into account the precise production dynamics of core BM components, we also mathematically model their synthesis and degradation rates allowing us to predict BM turnover.

Results

We show that migrating macrophages (hemocytes) produce and deliver the majority of BM components throughout the embryo. Failure in this delivery leads to an uneven distribution of ECM, morphogenetic defects, and embryonic lethality. Furthermore, we reveal that hemocytes regulate their temporal expression of specific BM components to allow for their proper incorporation. Finally, mathematical modelling predicts that rapid turnover (half-life = ~17 hours) is critical to define the expression levels of BM components. Indeed, manipulating matrix metalloprotease (MMP) expression in the developing embryo alters Collagen IV expression dynamics as predicted by the model, and affects morphogenesis of the developing nerve cord, which is dependent on BM.

Discussion

Taken together, these results reveal that *de novo* BM construction *in vivo* requires a combination of both production and distribution logistics allowing for the timely supply and incorporation of core components. Moreover, the rapid turnover of the BM reveals that BM is a far more dynamic structure than previously thought.

P45, ST: The role of the circadian clock in the homeostasis of the extracellular matrix in cartilage and intervertebral discs

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Introduction
The circadian clock is a molecular mechanism that allows organisms to anticipate the daily changes in their activity driven by the night and day cycle. The rest/activity pattern of behaviour is reflected in tissues on the level of gene transcription, translation and protein degradation. We hypothesised that the circadian clock would be of particular importance in rhythmically loaded tissues, such as the cartilage and intervertebral discs (IVDs) that need to maintain their vast extracellular matrix (ECM) after bouts of daily activity.

Materials & Methods
We utilised real-time bioluminescence recording of PER2::Luc clock reporter mouse tissue explants to monitor the dynamics of the circadian clock in cartilage and IVDs. RNA sequencing and mass spectrometry was used to reveal the rhythmic transcriptome and proteome. *Col2a1* specific Bmal1 KO ("clockless") mouse model was used to investigate the consequences of disruption of the circadian rhythm.

Results and Discussion
We show the first evidence that cartilage and IVDs possess circadian rhythm and that it is affected by aging and disrupted by pro-inflammatory cytokines (Dudek et al JCI2016, Dudek et al ARD2017). Our unpublished results show that it is also responsive to daily changes in osmolarity of the ECM and to mechanical loading. Time series RNAseq of mouse cartilage and IVDs revealed hundreds of tissue specific rhythmic genes. Circadian disruption by deletion of Bmal1 in Col2a1 expressing cells resulted in loss of rhythmic gene expression and dysregulation of key metabolic pathways. Time series mass spectrometry of WT mouse hip cartilage shows for the first time that cartilage proteins, including dozens of ECM proteins, exhibit a diurnal pattern of abundance. Histological analysis of the Bmal1 KO mouse phenotype revealed age dependent degeneration of the knee cartilage and fibrosis and calcification of IVDs. Taken together our data suggest that the circadian rhythm is an essential modulatory mechanism for cartilage and IVD physiology, regulating gene expression and protein abundance on a daily basis. Moreover, osmotic and mechanical signalling is involved in setting the pace of the musculoskeletal circadian clock. Coupling of metabolism with the activity pattern allows these tissues to efficiently synthesise or remodel their ECM at the right time and maintain tissue homeostasis.

P46, Secretion of TGFb1 by fibroblasts and macrophages is executed by secretory autophagy

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TGFb1 is a pleiotropic cytokine with cell type-specific effects modulating growth, survival and differentiation. It plays key roles in tissue fibrosis, inflammation and tumorigenesis.
TGFb1 is synthesized as small latent complex (SLC), consisting of a LAP (latency-associated peptide) prodomain and the mature growth factor. SLC is tethered to latent TGFβ binding protein (LTBP) to form the large latent complex (LLC). Only this complex is efficiently released to the extracellular space where it is sequestered by anchorage to fibrillin microfibers in the extracellular matrix (ECM). Fibroblasts are key cells depositing ECM and their function crucially depends on autocrine TGFβ1 signaling. While signaling from different TGFb receptors and extracellular activation of TGFb1 are well understood, information on its intracellular trafficking and secretion is sparse but has important biological and clinical implications. We identified secretory autophagy/regulated secretion as the molecular pathway for TGFb1 release from human and murine fibroblasts and macrophages.
We found LAP-TGFb1 to co-localize with GRASP55, involved in selecting cargo for trafficking in specialized Golgi-derived vesicles. These structures were also positive for the autophagosomal marker LC3B, and moreover, EM analysis detected LAP-TGFb1 in autophagosomes. Immunoprecipitation revealed that GRASP55 directly interacts not only with LC3B but also with other mammalian ATG8-like proteins via an LC3-interacting motif (LIR). Depleting GRASP55 or mutating the LIR motif severely impaired TGFb1 secretion, suggesting that TGFb1 secretion may require formation of a complex involving GRASP55, LC3 and LAP-TGFb1. Of note, abrogating autophagosome formation by ablating ATG5, ATG7 or Beclin-1 or chemical inhibition of autophagy effectively blocked TGFb1 secretion in murine and human fibroblasts and macrophages, underscoring the crucial importance of autophagosome formation for TGFb1 secretion. TGFb1 containing secretory autophagosomes are transported to the plasma membrane in a mechanism depending on RAB8A. It is unclear at present how TGFb1 is released from its carriers to bind to ECM structures, and whether the other TGFb isoforms or members of the large family of TGFb proteins are also released by secretory autophagy.
The regulated secretion of this potent cytokine through the unconventional autophagy-dependent mechanism adds another level of control to TGFβ1 bioavailability and may open novel therapeutic options.

P47, The role of circadian clock genes in regulating the chondrogenic potential of human pluripotent stem cells

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Introduction

Osteoarthritis is a disease that results in the progressive loss of articular cartilage. Human pluripotent stem cells (hPSCs) differentiated towards a chondrocyte lineage may provide a scalable source of cells for a regenerative therapy. The circadian molecular clock is a fundamental component in the homeostasis of tissues such as cartilage. hPSCs do not appear to maintain a circadian clock until it is activated at a certain point during differentiation. The aim of this study is to determine exactly how and when the cell intrinsic circadian molecular clock is activated during chondrogenic differentiation of hPSCs. Moreover, can modulation of the molecular clock be used to enhance chondrogenic outcomes.

Materials and Methods

hPSCs expressing the reporter mPer2::Luciferase were directed towards a chondrocyte lineage using a defined 14 day 2D differentiation protocol. Post day 14, cells were cultured in 3D pellet culture for a further 28 days. Protocol cultures were assessed using bioluminescence microscopy at different points throughout the protocol to determine the point at which the circadian molecular clock is activated. In addition, a juvenile chondrocyte cell line (TC28a2), which displays an active circadian molecular clock, were cultured in 2D and 3D for comparison.

Results

Initial results show that circadian rhythm is indeed absent in hPSCs and is subsequently activated during the 3D phase of chondrogenic differentiation. In addition, the 3D culture phase of the differentiation protocol produces extracellular matrix (ECM) structures architecturally comparable to those seen in histological analysis of cartilage.

Discussion

The activation of the mPer2::luciferase reporter during the 3D stage of chondrogenic differentiation suggests that this model may be used to evaluate the currently unknown mechanisms leading to the activation of the circadian molecular clock. Moreover, the production of ECM structures comparable to those seen cartilage may be used to investigate the influences of circadian controlled gene transcription on pellet ECM composition.

P48, Collagen is not just collagen – differential matrix expression induced by TGF-β and PDGFs

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Introduction
Accumulation of extracellular matrix (ECM) proteins is the hallmark of fibrosis, which can lead to altered tissue homeostasis, organ failure and ultimately death. Many different cell types and growth factors are involved in this process but fibroblasts are the main source of ECM proteins. Here we present results from an in vitro model indicating that transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF)-AB and BB induce synthesis of different ECM proteins relevant for pathogenesis.

Materials and Methods
The effect of TGF-β and PDGFs on ECM protein synthesis was assessed in a scar-in-a-jar (SiaJ) cell model using human renal fibroblasts. Cells were seeded in 48-well plates at 30.000 cells/well and incubated for 24H in DMEM + 10% FBS. Serum starvation was done by seeding the cells for further 24H in DMEM + 0.4% FBS. Fresh medium was added at day 0 with 225/150mg/mL Ficoll 70/400 and 1% ascorbic acid, containing 0.04 nM TGF-β, 4-, 0.4-, or 0.04 nM PDGF-AB or -BB or a vehicle control. Medium was changed and collected at day 3, 6, 10 and 13. Biomarkers of collagen type I (PINP), III (PRO-C3), VI (PRO-C6) and fibronectin (FBN-C) formation were assessed in the medium.

Results
TGF-β induced a significant increase in PINP, PRO-C3 and FBN-C compared to PDGFs and the vehicle. Levels increased more than 50-fold for PINP and FBN-C and 10-fold for PRO-C3 compared to vehicle. PDGFs increased FBN-C and PINP but not PRO-C3 compared to the vehicle, but with lower potency than TGF-β. PRO-C6 was inhibited by TGF-β and dose-dependently stimulated by PDGFs. FBN-C and PINP peaked after 6 days of TGF-β treatment, while PRO-C3 and PRO-C6 peaked after 10 days of TGF-β and PDGF stimulation, respectively.

Discussion
These data provide insight in the complex regulation of ECM protein synthesis induced by growth molecules, and show that different growth factors induce different protein expression profiles in fibroblasts. Collagen synthesis is thus regulated differentially. This SiaJ model in combination with the investigated biomarkers of ECM formation could be used to elucidate the mechanisms behind acute and sustained matrix production profiles. Ultimately, this could point toward novel therapeutic intervention points in fibrogenesis.

P49, Developing an *in vitro* human induced pluripotent stem cell (hiPSC) model using CRISPR/Cas9 to investigate the role of perlecan in fibrosis

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Introduction

Perlecan is a modular heparan sulphate proteoglycan of basement membranes and the basal laminae. The N-terminal domain of the protein core, domain I has three glycosaminoglycan attachment sites decorated with heparan sulphate. The C-terminal domain, domain V contains an 12 integrin binding site and glycosaminoglycan attachment site that can be decorated with chondroitin sulphate. This domain has been implicated in the fibrosis associated with renal allograft transplantation, but the mechanism of action is not clear. Domain III has laminin-like domains and has been implicated in cell adhesion with the precise mechanism not yet elucidated. This project aims to use the CRISPR/Cas9 gene targeting system to specifically target these domains so that we can investigate their roles in fibrosis.

Materials and Methods

CRISPR/Cas9 targeting plasmids were designed and constructed with Snapgene™ using the genomic perlecan (*HSPG2*) sequences from the UCSC server (https://genome.ucsc.edu/) and Pubmed™. Guide plasmids were designed using the CRISPR guide design site at MIT (http://crispr.mit.edu/). hiPSCs were transfected and clones selected through puromycin/blasticidin resistance. The expression of perlecan was assessed using immunocytochemistry and domain specific antibodies together with qPCR and domain specific primer sets.

Results

We have constructed a knockout plasmid targeting the start of exon 2 of *HSPG2* by introducing a premature stop codon. After transfection into hiPSCs and selection, we isolated heterozygous clones in which one allele was disrupted. These have reduced perlecan mRNA (~50% by qPCR), and protein expression by immunofluorescence. Targeted clones maintained pluripotency and capacity to differentiate into cardiomyocytes, demonstrating that a reduced expression of perlecan had no significant effect on the ability of hiPSCs to respond to differentiation cues.

Discussion

Our results show that we can use CRISPR/Cas9 gene targeting to successfully target the perlecan gene (*HSPG2*) to create clonal hiPSC lines with reduced expression, but maintain differentiation ability. Next, we plan to create a homozygous perlecan deletion and hiPSC lines that have been modified to express truncated forms of perlecan lacking either domain III or V. These cell lines will be used to investigate ECM composition and secretion as the cells differentiate into cardiomyocytes and respond to fibrotic stimuli.

P50, Co-ordination of mechanotransduction by talin and vinculin

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Introduction
Cells continuously probe and respond to biochemical and mechanical properties of their surroundings. The extracellular matrix (ECM) is an important part of the cellular surrounding and cells sense it through transmembrane adhesion receptors (integrins). In focal adhesions (FAs), integrins are linked to the contractile actin cytoskeleton via a large number of FA plaque proteins that dynamically regulate this link. Of particular interest are the proteins talin and vinculin, which connect integrins to the actin cytoskeleton.

Materials and Methods
We use photokinetic microscopy (FLAP) to measure protein turnover within FAs on engineered polyacrylamide substrates of different stiffness. To investigate mechanisms of talin-vinculin interactions and how these contribute to mechanosensing, we targeted either protein to a force-free environment at the mitochondria, allowing us to study how forces contribute to protein activation.

Results
Our findings suggest that FAs are built of different modules: one comprising the mechanosensing adaptor proteins talin and vinculin; the other containing signalling proteins such as FAK and paxillin. We show that the mechanosensing proteins are involved in rigidity sensing, whereas the signalling proteins control downstream signalling to influence the actin cytoskeleton. Using a number of talin and vinculin mutations we demonstrate how they mutually interact, and show that active vinculin can bind to talin independently of forces, and *vice versa*. Mechanistically, this is facilitated by relief of talin auto-inhibition, which may act to destabilise the main vinculin-binding region with talin.

Discussion
Together our data suggest that FAs are composed of functional modules that separately control the events of mechanotransduction, which involves both mechanosensing and mechanosignalling. Talin-vinculin interactions can occur independently of forces, although forces are required for mechanosensing.

P51, Laminin-332 in the progression of cutaneous squamous cell carcinoma – 3D cell culture system as an *in vitro* tumor model

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Introduction
Cutaneous squamous cell carcinoma (cSCC) is the most common metastatic skin cancer. At present, no validated biomarkers are available for identification of cSCCs that progress to invasive or metastatic carcinomas, and thus, the prognosis of metastatic cSCC is poor. To investigate the progression of cSCC, we have established a 3D cell co-culture model (spheroids) by using human keratinocyte-derived cell lines and human primary skin fibroblasts.
Altered expression of basement membrane protein Laminin-332 has been linked to many carcinomas, including SCC. We show that in cancer cells, Laminin-332 expression is increased by fibroblasts, which are also crucial for cancer cell invasion. In addition, we connect Laminin-332 expression to oncogenic H-Ras signaling.

Materials and Methods
HaCaTs and H-Ras-transformed HaCaT human keratinocytes (A5, II-4 and RT3) were cultured with or without human primary skin fibroblasts in 3D spheroids (3D Petri Dish, MicroTissues). Mass spectrometric analysis, Western blotting and immunofluorescence stainings were performed to detect Laminin-332 expression in 3D spheroids. H-Ras silencing was done by siRNA transfection. Invasion assays were conducted by using collagen I gel (bovine skin collagen I, Nutragen).

Results
The organization of HaCaT/HaCaT-ras human keratinocytes reflected their tumorigenity in 3D co-cultures with primary fibroblasts. Mass spectrometric analysis and Western blotting from the same co-cultures showed increase in Laminin-332 expression compared to monocultured spheroids. The expression was dependent on oncogenic H-Ras, since H-Ras silencing in cancer cells completely blocked Laminin-332 expression in co-cultured spheroids. However, this did not occur in traditional 2D cell culture conditions. In addition, cancer cell invasion from 3D spheroids occurred only when they were co-cultured with fibroblasts.

Discussion
Our data show that *in vitro* 3D cell culture model is a valuable tool for exploring the progression of cSCC. We demonstrate differential Laminin-332 expression in 2D and 3D cell cultures, highlighting the importance of more physiologically relevant, multicellular 3D cell culture models in cancer research. We show that Laminin-332 expression in cancer cells is considerably increased by a soluble factor released by primary skin fibroblasts, and the expression is linked to oncogenic H-Ras signaling. In addition, the cancer cells invaded only in the presence of fibroblasts. Our results emphasize the indispensable role of ECM proteins and fibroblasts in cancer cell invasion and metastasis.

P52, Perineuronal chondroitin sulfates reinforce inhibitory GABAergic transmission in the maturation of sensori-motor function

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Introduction
The last steps in the maturation of sensori-motor functions are often subject to experience-dependent modulations. In rats, we observed onset of negative geotaxis (an innate behavior indicative of graviception) as from postnatal day (P)9, time-matched with the consolidation of chondroitin sulfate (CS)-rich perineuronal nets (PN) around GABAergic/parvalbumin-expressing neurons in the vestibular nucleus (VN). Experience-dependent plasticity was revealed when bilateral labyrinthectomy in neonates significantly deterred both the onset of negative geotaxis and the consolidation of PN-CS around GABAergic interneurons in the VN.

Materials and Methods
Using SD rats as model, negative geotaxis and air righting were investigated as behavioural readout of graviception. Whole-cell patch-clamp recordings were performed on VN interneurons in brainstem slice at P9 and P14 to investigate spontaneous inhibitory and excitatory post-synaptic GABA or AMPA receptor-mediated current (sIPSC and sEPSC) and EPSC/IPSC ratio.

Results and Discussion
In labyrinth-intact rats, treatment of the VN at P6 with chondroitinase ABC (ChABC) resulted in loss of PN-CS. The effect lasted up to P13 during which developmental onset of negative geotaxis was delayed to P13. Is PN-CS critical for hardwiring inhibitory GABAergic transmission in the postnatal vestibular circuit? With whole-cell patch-clamp recordings performed on VN interneurons in brainstem slice preparations at P9 and P14 when PN-CS underwent consolidation, we found significant increase in frequency of sIPSC. Pre-treatment of the VN with ChABC at P6 led to 50% reduction in frequencies of IPSCs both at P9 and P14 but no accompanying change in amplitude of sIPSC. After acute ChABC treatment, significant increase of EPSC/IPSC ratio for frequency but not for amplitude per sampled neuron was likewise observed. In contrast, neither frequency nor amplitude of sEPSC showed significant change in this period. Support is thus provided for a role of PN-CS in reinforcing inhibitory/GABAergic inputs to VN interneurons in the maturation of the vestibular pathway for the graviceptive behaviour.
Acknowledgement: The project is supported by HKRGC-GRF 17125115 and HKU Seed Funding for Strategic Interdisciplinary Research Scheme.

P53, An optimised sample preparation and analysis workflow for expansion of the human bone proteome

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Introduction

Proteomic analysis of bone is technically challenging, primarily due to difficulties associated with tissue extraction. The inorganic matrix of bone hinders efficient extraction of protein, and methods that are commonly applied can leave residual insoluble protein, which is usually excluded from downstream analysis.

Existing efforts to characterise the bone proteome have almost exclusively utilised trypsin to digest extracted proteins, prior to 'shotgun' mass spectrometry (LC-MS/MS) analysis. This use of a single enzyme limits the possible coverage of the proteome. Furthermore, 'terminomics' methods used to reveal evidence of endogenous proteolytic processing, have not yet been applied to the investigation of bone. As such, the bone proteome remains poorly characterised relative to other tissues.

We have developed a sample preparation and analysis workflow for proteomic analysis of human alveolar bone, which addresses these issues and expands the known bone proteome.

Materials and Methods

Healthy human alveolar bone was obtained as surgical discard in accordance with a protocol approved by the University of British Columbia Research Ethics Board. We optimised existing bone protein extraction protocols, then digested fractions of extracted proteins using 4 different proteases prior to LC-MS/MS analysis. We optimised search parameters to include post-translational modifications commonly present in bone, and searched data with multiple search engines in order to increase the confidence of matched spectra. We also performed Terminal Amine Isotopic Labelling of Substrates (TAILS) analysis of bone protein extracts, to enrich for and detect endogenous neo N-termini.

Results

We identified >2300 proteins in bone at 1% false discovery rate. This number included 'missing' proteins, for which conclusive evidence at the protein level did not exist prior to this study. TAILS analysis established the first human bone n-terminome, detecting endogenous proteolytic processing of extracellular matrix proteins.

Discussion

Our identification of 'missing' proteins expands the known human proteome. The peptide modifications and endogenous N-termini that we detected provide an insight into the post-translational processing of proteins in bone. Our methods and data will facilitate future proteomic analysis of bone in human disease and transgenic animal models.

P54, Characterization of AMACO and other basement membrane associated Fraser complex proteins

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Introduction
AMACO (VWA2 protein) is a basement membrane associated protein containing VWA-like domains. It is expressed in skin, developing teeth, kidney, choroid plexus and was found as a molecular signature for hair placodes. AMACO is strongly expressed when invagination or budding occurs during development. The function of AMACO could be to mediate contact between epithelial and underlying mesenchymal cells. AMACO is part of the Fraser complex (FC). Fras1/Frem proteins each contain 12 CSPG (chondroitin sulphate proteoglycan) repeats and thereby form a distinct subgroup of the Fraser complex proteins. Loss of expression of FC components like Fras1 or Frem proteins causes Fraser syndrome, in which cohesion between epithelial tissues and stroma is perturbed.

Materials and Methods
Frem1, -2 & -3 CSPG repeats were cloned using mouse cDNAs from embryo, brain, kidney and ovary. The recombinant protein secondary structures were established with circular dichroism experiment. Protein interaction was demonstrated by surface plasmon resonance and confirmed by ELISA style binding assays. The recombinant Frem2 protein was later used to produce an affinity purified antibody. We studied the architecture of the FC in embryonal skin by immunoelectron microscopy using gold-labelled antibodies.

Results
Frem2 and -3 CSPG repeats were successfully expressed in eukaryotic cells. While Frem3 CSPG repeats were retained in the cells, Frem2 CSPG repeats were however secreted into the supernatant and therefore produced on a large scale. The Frem2 CSPG repeats form predominantly a monomer, are properly folded and contain mainly β sheets. CSPG repeats of Frem2, like those of Fras1, also interact with AMACO. AMACO binds to the CSPG repeats of Fras1 and also to nephronectin. AMACO transiently forms previously unknown, extended cable-like suprastructures in the dermis that was observed also in human fetal skin (GW 21).

Discussion
AMACO and Frem2 were further characterized as members of the Fraser complex. Although they are expressed by keratinocytes, cables of about 60 nm originate at the dermal side of the basement membrane and often end at the cell surface of fibroblasts. The binding partners Fras1 and nephronectin have a more restricted distribution which only partially overlaps. We have recently started to characterize an AMACO KO mouse to study its function.

P55 The role of cryptochrome proteins in the regulation of TGF-β1 signalling pathway and their implication in tissue fibrosis

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Introduction

Chronic tissue scarring resulting from unresolved tissue repair contributes towards the leading causes of morbidity and mortality worldwide. One major research focus has been the analysis of the molecular pathways that control extracellular matrix synthesis (ECM). The circadian clock is an endogenous timing system that allows an organism to anticipate and optimally respond to daily changes in the environment over a 24h day/night cycle. Human and mammalian tissues and cells have an intrinsic biological clock, driven by a conserved set of core genes and proteins that form positive/negative feedback loops. Recent studies demonstrated that genetic clock disruption in mouse models lead to increased susceptibility of tissues (lung, liver, kidney) to fibrotic injury. Here, we investigated the role of the negative loop components of the clock, cryptochrome (CRY) proteins, in the regulation of ECM genes implicated in fibrosis.

Materials and Methods

The expression of several matrix genes and fibrotic markers was assessed in peripheral tissues from wild type (WT) mice over 24h using qPCR. Primary mouse embryonic fibroblasts (MEFs) from WT and Cry1/2 KO mice (a genetic model of arrhythmic clock) were used to test the expression and rhythmicity of matrix genes following clock-synchronisation. Pharmacological Cry1/2 stabilisation or Cry1/2 plasmid over-expression were used to assess matrix genes following TGFβ stimulation. Matrix gene promoter activity was assayed using dual luciferase reporter assays. Cry1/2 expression was assessed over 24h in mouse models of fibrosis.

Results

We show rhythmic expression of several matrix genes implicated in fibrosis in the lung and kidney of WT mice. They are regulated, in part, by BMAL/CLOCK responsive elements in their gene promoters. Elevated endogenous expression of target matrix genes was observed in Cry1/2 KO MEFs. TGFβ stimulation led to further induction of matrix genes in Cry1/2 KO MEFs, which was prevented using canonical TGFβ pathway inhibitors or WT matrix rescue. Pharmacological stabilisation of CRY proteins reduced TGFβ-stimulated induction of matrix genes in WT MEFs. Finally, we observed downregulation of Cry1/2 genes and their dampened rhythmicity in the lung and kidney of fibrotic mouse models.

Discussion

These results demonstrate that *Cry* represses expression of target matrix genes in a TGFβ-dependent manner and that fibrotic conditions lead to reduced *Cry* rhythms. Thus, *Cry* may play an important role in the negative feedback regulation of matrix genes during resolution of tissue repair.

Workshop 4: ECM Microenvironment, Adhesion and Cell Fate

P56, Investigating the roles of perlecan using antibodies with unique epitopes

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Introduction
Perlecan is a major heparan sulfate proteoglycan of basement membranes. The core protein is divided into five domains with each domain involved in modulating various cellular processes through interactions with extracellular matrix molecules such as laminins as well as growth factors and cytokines. With such an integral role in regulating cell adhesion and proliferation to vascular development and immune function, perlecan is being studied for its role in tumorigenesis and its potential as a novel target for therapeutic intervention.

Materials and Methods
Using hybridoma technology, we have generated a panel of monoclonal antibodies that bind human perlecan. The binding specificity of the antibodies to full-length perlecan was confirmed by enzyme immunosorbent assay (ELISA) and Western blotting. The antibodies have been epitope binned using surface plasmon resonance (SPR) technology by screening the antibodies against recombinantly expressed perlecan domains. Having identified anti-perlecan antibodies with unique epitopes, the antibodies were investigated for their functional properties of modulating endothelial, fibroblast and cancer cell line adhesion, proliferation and migration in a series of cell-based assays.

Results
The antibodies have been organised into distinct bins based on their binding profile to individual domains and their ability to competitively block or sandwich pair with one another. Anti-perlecan antibodies that specifically bind to domains I, III and V have been identified. The binding specificity correlated to functional activity with domain-specific antibodies modulating or inhibiting adhesion, proliferation and migration of model cell lines used to reflect the main cell types localised in the tumour stromal environment including endothelial (HUVEC), fibroblast (MRC-5) and cancer (colon: WiDr) cells.

Discussion
These studies have demonstrated the significance of epitope mapping antibodies prior to the downstream assessment of functional activity. The binding specificity to perlecan domains combined with functional screening has provided insight into the functional relevance of the individual domains and their interacting molecules to elucidate the roles of perlecan in biology and pathology.

P57, Fine structural modifications of heparan sulfate sulfation patterns in lung are associated with functional effects in Precapillary Pulmonary Hypertension

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Introduction

Sulfated glycosaminoglycans (GAG), heparan (HS) and chondroitin (CS) sulfates, are essential components of the extracellular matrix (ECM) in lung homeostasis. In pulmonary hypertension (PH), altered ECM participates to lung arterial remodeling. We hypothesized that changes in GAG patterns, composition and fine structure could alter their selective effects on growth factors binding and cell properties, thus leading to vascular remodeling in lung PH. Here we deciphered the fine structure and bioactivity of GAGs both in human and rat experimental PH.

Materials and Methods

We performed quantitative (GAG total amount, HS/CS ratio), qualitative (disaccharidic sulfation pattern) and functional analysis (growth factor binding, cell proliferation) of HS and CS extracted from human lungs of patients with precapillary PH and controls, and of monocrotaline PH rats. Patients with idiopathic (iPAH), heritable (hPAH) PAH, or pulmonary veno-occlusive disease (PVOD) were included. Both human and animal studies were approved by ethical committees.

Results

Lungs of patients with iPAH, hPAH and PVOD revealed specific HS accumulation associated to selective HS disaccharides sulfation patterns. Monocrotalin rats presented increased GAGs/mg lung, preceding vascular remodeling, together with changes in disaccharides sulfation patterns, suggesting early alteration of GAG structure in disease progression. These changes were associated to significant functional modification of GAG binding affinity to growth factors (VEGF, FGF-2, PDGF), and significant induced cell proliferation in the presence of altered GAGs and growth factors.

Discussion

We suggest that changes of GAG composition and HS sulfation patterns in the lung could maintain a chronic inflammatory and vascular remodeling process leading to deregulated vascular cell proliferation through abnormal binding to growth factors. Further studies are focusing on new glycanic targets in pathological lung for future therapeutic intervention.

P58, Modification of the extracellular matrix of the arterial wall by myeloperoxidase contributes to atherosclerosis

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Introduction
The extracellular matrix (ECM) of the vascular basement membrane is vital for maintaining the functional and mechanical properties of arteries. The structure and properties of this material are altered during the development of atherosclerosis. The oxidant-generating enzyme myeloperoxidase (MPO) is known to be present in human atherosclerotic lesions, and the concentration of this enzyme correlate with disease severity and outcomes. This study examined the hypothesis that hypochlorous acid (HOCl), a powerful oxidant generated by MPO at micromolar concentrations, affects native human coronary artery smooth muscle cell (HCASMC)-derived ECM, and that these modifications modulate HCASMC behaviour and phenotype, thereby contributing to progression of atherosclerosis.

Materials and Methods
Native HCASMC-derived ECM was harvested and exposed to increasing concentrations of HOCl (0-200 μM, both reagent and generated by MPO) and the effects on ECM proteins investigated using SDS-PAGE and antibodies against specific ECM proteins via ELISAs and Western blots. Modulation of HCASMC adhesion and proliferation was examined using calcein-AM and MTS. mRNA was isolated from HCASMC exposed to HOCl-modified ECM to investigate effects of modified ECM proteins on genes associated with inflammation, ECM protein synthesis and turnover, via quantitative real-time PCR.

Results
HCASMC-ECM exposed to increasing concentrations of HOCl (0-200 μM) resulted in changes in structure and a loss of antibody reactivity against the fibronectin cell-binding fragment (CBF), laminins, type IV collagen and versican G1 domain. Exposure of HCASMC to ECM pre-treated with > 10 μM HOCl, resulted in concentration-dependent reduction in HCASMC cell adhesion and proliferation. mRNA expression of HCASMC genes associated with the inflammatory response (*IL-6*, *COX-2*), matrix protein synthesis (*FN1*) and turnover (matrix metalloproteinases; *MMP1*, *MMP11*, *MMP13*), were up-regulated by ECM pre-treated with > 1 μM HOCl.

Discussion
These data show that HOCl induces structural and functional damage to HCASMC-ECM proteins, which subsequently can modulate HCASMC cell adhesion and proliferation. Our results reveal a novel pathway through which enzyme (MPO)-induced oxidation modifies ECM components, and contributes to behavioural switching of HCASMCs, a key process during the progression of atherosclerosis.

P59, Influence of *Ruta graveolens* 9CH on murine melanoma progression

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Introduction
Dynamic and reciprocal interactions generated by the communication between tumor cells and their matrix microenvironment, play a major role in tumor progression. Adhesion of specific sites to matrix components associate to formation of membrane protrusions, allow tumor cells to move along a determined pathway. Today, some conventional treatment for cancer, are often unable to provide the expected response. In order to improve their effectiveness, there are a rising demand in complementary and alternative medicine. Among them, homeopathy is already known to have effects on some tumor cells, particularly the *Ruta graveolens* specific homeopathic strain. In this study, the mechanism of action of *Ruta graveolens* 9CH was studied about tumor process on a specific matrix environment, to explore new therapeutic approaches in cutaneous melanoma.

Materials and Methods
B16F1 and B16F10 murine melanoma cells were seeded on fibronectin and treated with *Ruta graveolens* 9CH. Cells dispersed migration and cells circularity were analyzed for 24h using time-laps videos. Cells stiffness (atomic force microscopy), membrane structure changes (confocal microscopy using Laurdan fluorescent probe) and actin cytoskeleton (immunofluorescence assay) were explored.

Results
We demonstrated that *Ruta graveolens* 9CH treatment reduces significantly and sustainably B16 cells dispersed migration by 30% by altering development of cell protrusions. Moreover, *Ruta graveolens* 9CH greatly decreases cell stiffness on peripheral areas. Concomitantly, a disruption of actin filaments located just under the plasma membrane is observed from 1 h of treatment. Lastly, this homeopathic drug could alter plasma membrane structure by accumulating large ordered lipid domains.

Discussion
In conclusion, this study allowed us to demonstrate that *Ruta graveolens* 9CH disrupted *in vitro* murine melanoma cells migration by likely disrupting the balance between ordered and disordered lipid domain of plasma membrane. Whereas the correlation between lipid raft and cytoskeleton disrupting is not well established, *Ruta graveolens* 9CH may act on actin cytoskeleton organization, as evidenced by cell stiffness decrease, which ultimately fails to establish an effective migration process.

P60, ADAM12-mediated shedding of basigin controls the subcellular localization and lactate transport of MCT4 and enhances collagen degradation in cancer cells

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Introduction
Basigin (also named CD147/EMMPRIN), a transmembrane glycoprotein of the immunoglobulin superfamily, regulates the localization of monocarboxylate transporters (MCT)-1 and -4 at the cell surface, which allow the efflux of lactate produced by aerobic glycolysis. Moreover, basigin stimulates matrix metalloproteinase (MMP)-mediated extracellular matrix (ECM) degradation, driving cancer cell invasion. Basigin can be proteolytically shed from the cell surface and serum levels of soluble basigin in cancer patients often correlates with disease stage and poor prognosis. Interestingly, a positive correlation between basigin and a disintegrin and metalloprotease (ADAM)-12 in serum from prostate cancer patients was recently reported. Yet, the functional relevance of this correlation is unknown.

Materials and Methods
ADAM12 was overexpressed or knocked down in human cancer cells and basigin shedding was assessed by western blot. Immunofluorescent staining and microscopic analysis were used to determine protein localization and gelatin degradation. Database mining was performed to correlate gene expression and identify cancer-associated mutations.

Results
ADAM12 interacts with basigin and cleaves it in the juxtamembrane region. Overexpression of ADAM12 in human cancer cells induces endogenous basigin shedding, enhances the amount of MCT4 at the cell surface, and causes a significant increase in lactate efflux. Highlighting the relevance of this finding, RNAseq data show a statistically significant correlation between the expression of ADAM12 and MCT4 in human prostate tumors. Further database mining identified several cancer-associated point mutations in the basigin membrane proximal region. Interestingly, expression of identified basigin mutants in human cells showed that several of these are highly prone to ADAM12-mediated shedding and therefore more potently enhance the gelatinase activity of exposed cancer cells.

Discussion
ADAM12-mediated shedding of basigin may regulate the sub-cellular localization of MCT4 and consequent lactate efflux in cancer cells. Moreover, basigin shedding appears to be altered in some types of cancer, with potential implications for the ability of cancer cells to degrade the ECM and invade surrounding tissues.

P61, Vascular chondroitin/dermatan sulfate proteoglycans remodeling induced by ApoA- I and natural variants. Probable role in settlement of amyloidosis

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Introduction
Amyloidosis constitutes a heterogeneous group of diseases involving protein misfolding and deposition of fibrils. Apolipoprotein A-I (apoA-I), the main protein of plasma high-density lipoproteins (HDL), removes excess cell cholesterol and protects against atherosclerosis. Nevertheless, some natural variants (R173P) or their N-terminal fragments (IOWA, N-terminal peptide of G26R) of the native protein with structural disorder elicit their propensity to suffer misfolding or aggregation. Moreover, amyloidosis due to the protein with the native sequence has been described as diffuse protein aggregates in atherosclerotic plaques. Our previous reports suggest that specific interactions of apoA-I with glycosaminoglycans could elicit its retention and/or aggregation. Furthermore, recent studies indicate that protein cores of proteoglycans (PGs) may influence the type and modification patterns of the subsequently attached glycosaminoglycan chains. We hypothesize that mutations in human apoA-I may affect the core protein pattern expression of vascular chondroitin/dermatan sulfate PGs, modulating chemical changes in the glycosylation pattern which elicit extracellular apoA-I aggregation.

Materials and Methods
WT apoA-I and the amyloidogenic mutants IOWA and R173P were obtained by molecular biology techniques. Human umbilical vein endothelial cells (HUVEC) were treated with 1.5-50 µg/ml for 24hs. MTT, immunofluorescence of NFκB and zymographic analysis were used to evaluate endothelial activation. PG protein cores were quantified using RT-PCR for decorin, biglycan and versican.

Results
WT, R173P or IOWA (1.5 µg/ml) treatment did not modify cell viability, NFκB nuclear translocation and metalloproteinase-2 and -9 activities. Decorin expression was significantly decreased by WT and R173P, 10 and 6 folds respectively, when it was compared with no treated cells (control). Whereas biglycan was increased 4-fold by IOWA variant. And versican expression was only detected after R173P treatment.

Discussion
WT and the studied natural variants do not elicit an inflammatory response in our experimental model. Nevertheless, our results indicate substantial modifications in the profile of chondroitin/dermatan sulfate PGs core proteins, depending on the protein or peptide employed. Considering that glycosaminoglycans polymerization and modification by sulfation and epimerization are influenced by the protein core; changes in the profile of PGs might be involved directly or indirectly in the equilibrium between protein function and citotoxicity.

P62, Protein disulphide isomerase A3/ERp57 drives production of a matrix-rich secretome that stimulates cell adhesion and migration

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Introduction
The activities of extracellular matrix (ECM) proteins are mediated by molecular interactions at cell surfaces, in the pericellular milieu and inside the ECM. There is great interest to manipulate ECM composition for cell culture, bioengineering, or clinical applications. However, targeting of individual ECM proteins is complex, due to their abundance and multiplicity of binding interactions. Here, we identify Protein disulphide isomerase A3 (PDIA3/ERp57) as a candidate intracellular modulator of pro-adhesive ECM. PDIA3 is an ER-resident protein that effects post-translational disulphide bond formation. Prior characterised substrates include proteins with cysteine-rich domains and the ECM protein fibronectin

Materials and Methods
Starting from biotin ligase-based proximity interactome proteomics, mechanisms and functions of PDIA3 were studied by pharmacological inhibition, immunoblotting, confocal microscopy, cell-based assays, gene knock-out fibroblasts and tandem-mass-tag quantitative proteomics

Results
We identified a novel intracellular association between the matricellular glycoprotein, thrombospondin1 (TSP1), and protein disulphide isomerase A3 (PDIA3/ERp57). TSP1 and PDIA3 bind in vitro and colocalise in the endoplasmic reticulum of human dermal fibroblasts (HDF). Loss of PDIA3 function, either by pharmacological inhibition in HDF or in Pdia3-/- mouse embryo fibroblasts, (KO-MEF), resulted in increased extracellular TSP1 and decreased fibronectin, in correlation with reduced cell spreading, F-actin organisation and focal adhesions. Conditioned media (CM) of KO-MEF also supported poorly the spreading, F-actin organisation and 2D migration of breast cancer cells, compared to CM of wild-type MEF (WT-MEF). The cellular phenotypes of KO-MEF could be normalised by CM or ECM from WT-MEF. Rescue depended on PDIA3 activity in WT-MEF and on secreted heparin-binding proteins, yet extracellular fibronectin was insufficient for rescue. Proteomics and functional assays on the heparin-binding secretomes of WT-MEF and KO-MEF identified additional ECM and growth factor proteins up- or down-regulated in KO-CM, of which CTGF/CCN2 was required for the adhesion-promoting activity of WT-CM.

Discussion
PDIA3 in fibroblasts controls extracellular abundance of thrombospondin1 and specifically promotes production of an ECM-rich, pro-adhesive microenvironment. These findings implicate PDIA3 as a potential translational target for regulation of microenvironmental quality in fibrosis, breast cancer, or other pathologies.
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P63, Internal cleavage and synergy with twisted gastrulation enhance BMP inhibition by BMPER

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Bone morphogenetic proteins (BMPs) are essential signalling molecules involved in developmental and pathological processes and are regulated in the matrix by secreted glycoproteins. One such regulator is BMP-binding endothelial cell precursor-derived regulator (BMPER) which can both inhibit and enhance BMP signalling in a context and concentration-dependent manner. Twisted gastrulation (Tsg) can also promote or ablate BMP activity but it is unclear whether Tsg and BMPER directly interact and thereby exert a synergistic function on BMP signalling. Here, we show that BMPER binds to Tsg through the N-terminal BMP-binding region and this N-terminal region of BMPER is also a better inhibitor of BMP-4 signalling than full-length BMPER. Furthermore, BMPER and Tsg cooperatively inhibit BMP-4 signalling suggesting they act in concert to dampen BMP activity. Furthermore, full-length BMPER, but not the N-terminal region, binds heparan sulphate proteoglycans at the cell surface, mediated by the C-terminal region of BMPER. Small-angle X-ray scattering and electron microscopy show that BMPER has an elongated arrangement allowing the N-terminal BMP-binding and C-terminal cell-interactive regions to be spatially separated. To gain insight into proteolytic processing and thereby the regulation of BMPER bioavailability, a disease-causing BMPER point mutation, P370L, previously identified in the acid-catalysed internal cleavage site, was introduced. The mutated protein was secreted but the mutation prevented intracellular cleavage. However, mutant BMPER was subsequently cleaved extracellularly at a downstream site, presumably at a cryptic matrix protease cleavage site. These data suggest that localisation of BMPER at the cell surface reduces BMPER activity which may be ameliorated by the mutation.

P64, Hot Topic: Structural characterization of the ectodomains of the four human syndecans

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Introduction
Syndecans are transmembrane proteoglycans. Numerous studies have been performed to determine their roles in cell signalling but their structure have been poorly characterized. We report here the structural analysis of the ectodomains of the four human syndecans.

Materials and Methods
The ectodomains of syndecans (ED1-4) were expressed with a N-terminal 6xHis tag and a C-terminal Flag tag in *E. coli*, purified on Ni-NTA and anti-Flag M2 columns and analysed by circular dichroism with and without 2,2,2-trifluoroethanol (TFE) or guanidinium chloride. Their hydrodynamic radius (R_h) and their radius of gyration (R_g) were calculated by dynamic light scattering (DLS), and small angle X-ray scattering (SAXS, ESRF, Grenoble, France, MX-1841, MX-1920).

Results
The ectodomains of syndecans contain a significant amount of intrinsic disorder (69% for ED1 and ED3, 52% for ED2 and 44% for ED4), 15-25% of β-strands and less than 5% of α-helix. However, the amount of α-helix increases by 5-fold in ED4 and up to 11-fold in ED2 and ED3 in presence of TFE. A low amount of polyproline II helix is formed in presence of guanidinium chloride. The R_g/R_h ratio is higher than 0.8 for ED1 and ED2, which indicates that these ectodomains are extended. They are flexible according to the shape of their normalized Kratky plots. The Rh and Rg values of ED-3 suggests that it exists both as a monomer and a dimer respectively. ED1, ED2 and ED4 could adopt two conformations based on Ensemble modelling, the D_{max} ratio between both forms ranging from 1.2 (ED4) to 1.4 (ED1).

Discussion
The ectodomains of human syndecans are elongated and flexible and can partially fold into α-helix. They exist under two major conformations in solution, which might correspond to an active form, able to interact with its partners, and an inactive form. The structure of the complexes of ED1 and ED3 with anastellin are currently under investigation to test this hypothesis.

P65, ST: Development of human antibody against ADAM28, a key modulator of tumor microenvironmental factors in non-small cell lung carcinomas

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Introduction
ADAM28 (a disintegrin and metalloproteinase 28) is overexpressed by carcinoma cells in non-small cell lung carcinomas (NSCLCs) and plays a central role in cancer cell proliferation and progression by regulating tumor microenvironmental factors in cancers. To aim for new target therapy of NSCLC patients, we have developed and characterized human neutralizing antibodies against ADAM28.

Materials and Methods
The Human Combinatorial Antibody Library (HuCAL) was used for the screening of antibodies. Specificity of antibodies was analysed by immunoprecipitation, immunoblotting, epitope mapping and Biacore system. Effects of the antibody on cell proliferation and apoptosis in lung adenocarcinoma cell lines were examined by BrdU incorporation and DNA fragmentation ELISA, and IVIS imaging system was used to test the effects on lung metastasis in NOD/SCID mice.

Results
By screening HuCAL using phage display panning, we obtained two antibodies 211-14 and 211-12, which inhibited the activity of ADAM28. Antibody 211-14 recognized the junctional region between the cysteine-rich domain and the secreted-specific domain with a K_D value of 94.7 pM. Proliferation of lung adenocarcinoma cell lines with ADAM28 expression such as PC-9 cells was effectively inhibited by the antibody, but no effect was observed on H1975 cells with negligible ADAM28 expression. von Willebrand factor-induced apoptosis was promoted by the antibody treatment only in ADAM28-expressing cell lines. In mouse lung metastasis models, antibody 211-14 significantly reduced tumor growth and metastases of PC-9 cells and prolonged survivals in the antibody-treated mice compared with the control IgG-treated ones. Combination therapy of the antibody and docetaxel was more effective than that of bevacizumab (human anti-VEGF antibody) and docetaxel, and showed further elongation of survival time compared with monotherapy. No adverse effects were observed even after administration 10-fold more than effective dose of the antibody to normal mice or monkeys.

Discussion
Our data demonstrate that antibody 211-14 is a neutralizing antibody specific to ADAM28 and suggest that this antibody may be a useful treatment remedy for NSCLC patients.

P66, Binding stoichiometry of HSP47 to collagen depends on the collagen sequence

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Introduction
HSP47 – also known as SERPINH1 – is of outmost importance for the proper folding of collagen molecules and mutations in HSP47 can lead to defective collagen secretion and *Osteogenesis imperfecta*. On the other side in cases of excessive collagen secretion (e.g. fibrotic diseases) HSP47 was always found to be upregulated.
HSP47 was previously reported to recognise a specific motif on collagen helices (Gx[S/T]GxRGxx). Structural analysis of the collagen-HSP47 complex showed the importance of Asp_{385} which forms a salt bridge with the arginine on the collagen chain.
We identified an previously unknown high affinity binding site for HSP47 which introduces an additional phenylalanine in the binding motif (Gx[S/T]GxRG**F**x) [unpublished]; however, the exact mode of binding to this altered binding site was unknown.

Materials and Methods
We recombinantly expressed canine derived HSP47 and foldon peptides containing a high affinity binding sequence. The binding properties were tested using an ELISA-style-binding assay and bio-layer interferometry. To understand our findings structurally, we co-crystallised HSP47 with the corresponding collagen mimetic peptides.

Results
Binding assays showed an increased affinity for peptides having a GxRG**F** motif, and slight improvement for GxRG**L** motifs. Interestingly, modelling phenylalanine in our existing crystals structure lead to server steric clashes. Therefore, we successfully crystallised HSP47 in complex with synthetic, homotrimeric collagen peptides containing RGF and RGL sequences. Interestingly, both complexes crystallised in different crystals forms but as a 1:1 complex (collagen triple helix:HSP47), contrasting to our earlier structures showing a 1:2 complex.

Discussion
Preliminary analysis of the structures showed that HSP47 undergoes some minor rearrangements to form a pocket for the hydrophobic phenylalanine residue. The increased affinity most likely results from a gain of water entropy upon shielding of the aromatic residues. Interestingly, due to the staggered nature of the collagen helix, the bulky phenylalanine only allows a single HSP47 binding. At the second binding site the phenylalanine residue is positioned differently and cannot be accommodated in the above-mentioned pocket. This demonstrates that although HSP47 is in principle able to bind on two sides, the actual stoichiometry greatly depends on the collagen sequence.

P67, ST: Kindlin-1 regulates the mammary tumour cell secretome

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Introduction
The focal adhesion protein kindlin-1 has been shown to regulate TGFβ-induced epithelial-to-mesenchymal transition in breast cancer cell lines, which has been attributed to increased invasive capacity. Indeed, increased expression of kindlin-1 has been associated with increased risks of lung metastasis in breast cancer, but the mechanistic basis of this link is unclear.

Materials and Methods
To investigate further the role of kindlin-1 in metastasis, the kindlin-1 gene, *Fermt1*, was deleted from mouse mammary tumour cells using the CRISPR-Cas9 system. Isolated secreted proteins were analysed by label-free mass spectrometry and bioinformatic approaches. Integrin-dependent cell adhesion was measured on defined substrates and endothelial cells. Experimental metastasis assays were performed in a mouse model of mammary tumourigenesis in the presence of inhibitors of integrin-mediated adhesion.

Results
Proteomic analyses of extracellular proteins identified a network of kindlin-1-dependent secreted proteins that were linked to metastasis. Interrogating multiple patient cohort datasets, survival analyses of the kindlin-1-dependent secretome genes demonstrated that they were significantly more associated with lung metastasis than with other (non-lung) metastasis. The secretome network included the large glycoprotein tenascin-C, a lung metastasis regulator associated with poor overall survival of patients with breast cancer. Regulation of tenascin-C expression was dependent on the ability of kindlin-1 to bind and activate β integrins, and β1 integrin was required for the outgrowth of micrometastases. Furthermore, loss of kindlin-1 expression reduced pulmonary arrest and metastatic colonisation in an integrin-dependent manner.

Discussion
These results show that the kindlin-1-dependent secretome is associated with lung metastasis and is elevated in cells metastatic to lung. Kindlin-1 may, therefore, contribute to a metastatic niche in pulmonary metastasis. Kindlin-1 expression enhances the metastatic potential of breast cancer cells by modulating integrin activity and promoting tumour cell adhesion to the metastatic niche. Thus, kindlin-1 is a critical mediator of early lung metastasis of breast cancer.

P68, Using Bioprinting to investigate axon pathfinding for peripheral nerve regeneration

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Introduction

Neuronal networks are critical for many body processes, such as brain function, movement and sensing. These neural networks are established during development and depend on precise axon guidance mediated by extrinsic molecular cues and extracellular matrix (ECM) proteins. Following spinal cord (SCI) or peripheral nerve injury (PNI), axon regrowth is impaired, leaving individuals with compromised movement or, in case of SCI, paralyzed. Our key objective is to elucidate how different ECM proteins and chemical cues determine axon outgrowth and pathfinding, thereby permitting the development of novel biochemical micropatterned materials for enhanced nerve regeneration.

Materials and Methods

To examine neuronal behaviour under various conditions, inkjet printing, light induced photopatterning (from Alveole) and microstamping techniques were compared to create an *in vitro* neuronal guidance assay. Light induced photopatterning revealed to be the most precise and flexible tool to create patterns.
We produced Fibronectin (FN) and Laminin (LM) crossed line patterns as a competition assay. We used rat dorsal root ganglion (DRG) as a model system for peripheral nervous system (PNS) regeneration, and a mouse CNS derived catecholaminergic neuronal cell line (CAD) as a model of the central nervous system (CNS).

Results

Our results indicate that defined tracks of FN and LM are able to guide axons of CAD cells and influence their directionality. Additionally, axons that started on FN preferred to stay on FN which could indicate a preference for FN. This could be due to integrin recycling or a protein concentration dependent effect.
Furthermore, by establishing an algorithm for neurite tracking, data output was automated to give indication for directionality preferences. By performing live cell imaging technique, we will furthermore access the growth dynamics and speed of growth on these defined patterns.

Discussion

We showed a technique to create defined protein patterns by using light induced photo-patterning as a tool for axonal pathfinding assays. Future work includes testing different ECM proteins and guidance cues for their guidance capabilities on neurons from the PNS and CNS.
Knowledge about guidance abilities of defined biochemical cues will allow the design of micropatterned surgical nerve repair devices for *in vivo* testing.

P69, The potent inflammatory oxidant, peroxynitrous acid, modifies basement membrane laminins in human atherosclerotic lesions

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Introduction
The vascular basement membrane is rich in laminin, a trimeric extracellular matrix (ECM) protein consisting of α-, β- and γ chains. The C-terminus of the α chain interacts with specific integrins on cells and plays a critical role in cell adhesion and signalling. Binding sites for other ECM species including perlecan, collagens, fibronectin and nidogen are also present in specific domains. These interactions are perturbed in atherosclerosis, where activated neutrophils, monocytes and macrophages generate oxidants, including peroxynitrous acid (ONOOH), which can alter ECM composition and may contribute to endothelial cell (EC) dysfunction, ongoing inflammation and smooth muscle cell (SMC) infiltration and proliferation. We hypothesise that specific laminin isoforms synthesized by EC and SMC are important in maintaining an intact and functional ECM in healthy arteries, and that inflammation and ONOOH formation modify laminin domains in a manner that alters cellular behaviour and promotes atherosclerosis.

Materials and Methods
Laminin isoforms synthesized by primary human coronary artery endothelial (HCAEC) and smooth muscle (HCASMC) cells, and present in advanced human atherosclerotic lesions were characterized by SDS-PAGE and Western blotting. Structural changes to laminins induced by ONOOH was determined using ELISA, WB, UPLC and LC-MS. Effects of ONOOH on cell behaviour were examined using cell adhesion assays.

Results
The laminin isoforms generated by HCAEC or HCASMC, and present in their ECM, differ to those detected in advanced human atherosclerotic lesions. ONOOH-modified laminin was detected in advanced human lesions. Treatment of HCAEC or HCASMC ECM with ONOOH resulted in a > 50% loss of antibody recognition of the cell-binding epitopes, a dose-dependent loss of Tyr and Trp residues, and formation of the specific biomarkers, 3-nitroTyr, 6-nitroTrp and di-Tyr. These modifications have been mapped to specific sequences using LC-MS peptide mapping, with some present within functional domains. These changes were associated with a loss of HCAEC adhesion to the modified laminins.

Discussion
These data suggest that the laminin isoforms produced by HCAEC or HCASMC play specific roles in maintaining a functional and intact ECM environment in healthy arteries, and that this balance is perturbed in atherosclerosis as a result of ONOOH formation. These laminin modifications appear to have functional consequences for cell behaviour.

P70, Understanding the role of extracellular matrix remodelling in cancer progression and metastasis

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Introduction
Homeostasis of the extracellular matrix (ECM) is critical for correct organ and tissue function. Both the biochemical and biomechanical properties of the ECM contribute to modulating the behaviour of resident cells and are more than just passive bystanders. In tissue diseases such as cancer, the ECM undergoes significant change. These changes, driven by both tumour and stromal cells, feed into the pathological progression of the disease.

Materials and Methods
Cancer associated fibroblasts (CAFs) are the major ECM remodellers in cancer, and underpin the stromal desmoplastic response of almost all solid tumours. Lysyl oxidases, secreted by both cancer cells and CAFs are a family of secreted copper-dependent enzymes that post-translationally remodel the ECM through cross-linking collagens and elastin. To date, a functional role for lysyl oxidases has been reported in almost all solid tumours.

Results
Our work has shown that CAF-driven and LOX-mediated ECM remodelling are critical in modulating Src, FAK and Akt signalling activation, VEGF-driven angiogenesis, and nFATc1-mediated osteoclastogenesis in both breast and pancreatic cancer. Thus, ECM remodelling plays an important and crucial role in primary tumour growth and invasion; the generation of pre-metastatic niches; the progression of organ fibrosis and tumour desmoplasia; and can be used to stratify patients that will respond favourably to treatment with already approved clinical drugs, including bisphosphonates, and ROCK, Src, Akt, VEGF and FAK inhibitors. Furthermore, we have developed new approaches to visualise and study ECM remodelling in cancer. We have established a novel *in situ* decellularisation approach (ISDoT), which allows high-resolution fluorescence and second harmonic imaging, as well as quantitative proteomic interrogation of the 3D structure and spatial organisation of the ECM.

Discussion
Understanding at the molecular level how the changing ECM landscape facilitates tumour progression is an important step in the treatment of cancer.

P71, ST: Collagen prolyl 4-hydroxylases in collagen fibril formation and wound healing in mouse skin

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Introduction
One central enzymes in collagen biosynthesis is collagen prolyl 4-hydroxylases (C-P4Hs), which hydroxylates prolines in collagenous repetition domain (-Gly-X-Pro-) to 4-hydroxyproline. Vertebrate C-P4Hs are $\alpha_2\beta_2$ tetramers with three isoforms of the catalytic α-subunit, β-subunit being always a protein disulfide isomerase. Knockout of *P4ha1* gene is embryonically lethal, whereas knockout of *P4ha2* gene causes no obvious phenotype. First recognized patient mutations in P4HA1 cause congenital connective tissues disorder. Here, we have studied the role of C-P4H-I and C-P4H-II in skin and cutaneous wound healing.

Materials and Methods
Mutant mice (P4ha1+/+;P4ha2+/-, P4ha1+/-;P4ha2+/-, P4ha2-/-, P4ha1+/-;P4ha2-/-) were used in the study. Skin structure and collagen fibrils were studied with histological stainings and transmission electron microscopy (TEM). The thickness of dermis was measured, and the amount and thermostability of the skin collagen was determined. The diameter of collagen fibrils in the dermis was measured. The wound healing was studied by following the closure and inflammatory response of cutaneous wounds and the skin inflammatory response was further studied by inducing inflammation with TPA and analyzing the inflammatory cells and cytokine levels.

Results
The skins of P4ha2-/- and P4ha1+/-;P4ha2-/- mice revealed a thinner dermis, less collagen and decreased collagen fibril diameter compared to control mice. Also, capillary basement membranes were expanded in these mice. The melting temperature of P4ha1+/-;P4ha2-/- mice skin collagen was decreased. The wound healing study showed no differences in the speed of wound closure, but there was a stronger inflammatory response with P4ha1+/-;P4ha2-/- mice in the beginning of the healing process. TPA treated skins of P4ha1+/-;P4ha2-/- mice had stronger inflammatory response.

Discussion
The decreased enzyme activity causes decreased collagen fibril thickness and collagen amount and alterations in the capillary basement membranes in the skin. In addition, the inflammatory response is affected. Thus, our results show the central role of C-P4Hs in thermostable collagen fibril formation, tissue organization and ECM function in inflammatory response.

P72, Laminin N terminus α31 displays re-distribution during limbal activation and corneal wound repair, and influences corneal adhesion and migration

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Introduction
Laminin N terminus (LaNt) α31 is a relatively unstudied protein derived from the laminin α3 gene but structurally similar to netrins. LaNt α31 has, to date, been investigated only in 2D keratinocyte culture where it influences cell migration and adhesion, processes integral to wound repair. Here we investigated LaNt α31 distribution in ocular surface epithelium, during limbal stem cell activation, and corneal wound healing.

Materials and Methods
Human, mouse, and pig eyes, ex vivo limbal explant cultures, and alkali burn wounds were processed for immunohistochemistry with antibodies against LaNt α31 along with progenitor cell associated proteins. LaNt α31 expression was induced via adenoviral transduction into primary epithelial cells isolated from limbal explants, and cell spreading and migration analysed using live imaging.

Results
LaNt α31 localised primarily to the basal layer of the conjunctival, limbal and corneal epithelial cells. However, staining was non-uniform with apparent subpopulation enrichment. This distribution largely matched that of keratin 15, epidermal growth factor receptor, and p63α, and displayed similar increases in expression in activated limbal explants. During active alkali burn-wound repair, LaNt α31 displayed increased expression in limbal regions and loss of basal restriction within the cornea. Distribution returned to predominately basal cell restricted once the wounded epithelium matured. Cultured corneal epithelial cells expressing LaNt α31 displayed increased 2D area and reduced migration associated suggesting a functional link between this protein and key wound repair activities.

Discussion
These data place LaNt α31 in position to influence laminin-dependent processes including wound repair and stem cell activation.

P73, ST: *Laminin α4* deletion leads to impaired hematopoietic regeneration following irradiation-induced injury and accelerates the progression of acute myeloid leukemia

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Introduction
Both normal hematopoiesis and leukemia are regulated by a specialized microenvironments in bone marrow (BM), termed as hematopoietic stem cell (HSC) niches, which consist of cellular niche components, extracellular matrix proteins (ECM), growth factors and cytokines. While our knowledge of HSC niche in hematopoiesis homeostasis has been advanced, the impact of the niche, in particular ECM, on hematopoiesis regeneration under pathological conditions remains poorly understood.
Laminins are heterotrimeric ECM composed of α, β, and γ chains and are present in BM. The laminin α4 chain (LAMA4) is an active component for LAMA4-containing isoforms. It has been reported that the interactions of LAMA4 with their receptor integrin α6 are important for HSC homing. However, the role of LAMA4 in regeneration after hematopoiesis injury and during leukemia development is not known.

Materials and Methods
We here have used *Lama4*$^{-/-}$ mouse model, multi-color flow cytometry, confocal imaging, transplantation as well as an acute myeloid leukemia (AML) mouse model to investigate the impact of Lama4 loss on HSC niche maintenance and hematopoietic regeneration post-injury and during AML progression.

Results
Lama4 deletion resulted in the reduction of mesenchymal progenitor cells (MPC, CD45-TER119-CD31-CD44-SCA1-CD51+) and endothelial cells, but not mesenchymal stem cells (MSC, CD45-TER119-CD31-CD44-SCA1+CD51+) in adult mouse BM. Following sub-lethal irradiation, *Lama4*$^{-/-}$ mice displayed slower and incomplete recovery of platelets, mature myeloid cells and erythrocytes, which was accompanied with the reduced frequency of myeloid progenitors and the accumulation of megakaryocyte-erythrocyte progenitors and immature erythrocytes in the BM. These data suggest an important role of LAMA4 for hematopoiesis regeneration and megakaryocyte and erythrocyte maturation post-irradiation. Mechanistically, the delayed hematopoietic recovery might be associated with downregulation of *Il6* in the MSC and *angiopoietin-1* in the MPC of *Lama4*$^{-/-}$ mice. **Most importantly, by transplanting AML cells with MLL-AF9 fusion gene into non-conditioned *Lama4*$^{-/-}$ mice, we demonstrated that *Lama4* deletion in BM niche led to an earlier onset of AML. Moreover, loss of *Lama4* in BM microenvironment accelerated AML relapse after therapeutic hematopoietic cell transplantation.**

Conclusion
Altogether, our study suggests that LAMA4 is required for efficient hematopoietic recovery post-irradiation induced injury and inhibits AML progression.

P74, Syndecan-1: a new component of epithelial podosomes

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Introduction
During the epithelialization phase of wound repair, basal keratinocytes migrate, proliferate and maintain dynamic interactions with extracellular matrix (ECM). Laminin 332, known as a major adhesion substrate for keratinocytes contributes to skin re-epithelialization through its α3 chain C-terminal globular domains 4 and 5 (so-called LG45). Recent studies have suggested that LG45 induces expression of the pro-migratory matrix metalloproteinase MMP-9. As syndecan-1 was shown to participate in cytoskeleton dynamic through binding to the laminin LG45 domains, we analysed its potential involvement in MMP-9 expression.

Materials and Methods
Site-directed mutagenesis was applied to alter binding properties of a recombinant LG45 protein. PCR and gel zymography approaches were used to analyze MMP-9 expression in the conditioned medium and ECM of keratinocytes. Syndecan-1 expression was knocked down with siRNAs in human primary keratinocytes. In situ gelatin zymography was assessed and analysis of various labelled antigens was done by confocal microscopy.

Results
Our PCR analysis and zymography results revealed that syndecan-1 plays a role in LG45 induced MMP-9 expression and activation. Down regulating syndecan-1 expression in keratinocytes confirmed these findings and revealed that this phenomenon also occurred when cells were treated with TNFα or IL1β, two cytokines known to up-regulate MMP-9 expression. In situ zymography performed with primary keratinocytes revealed areas of digested gelatin resembling adhesion contacts underneath keratinocytes. Their number was increased in LG45-treated keratinocytes and their formation inhibited by MMP-9 inhibitors. Their deeper analysis by confocal microscopy revealed syndecan-1 staining as bright rings surrounding a core of actin, cortactin, ARP2/3 and WASP localized within the digested gelatin. This data suggests that these clusters belong to epithelial podosomes.

Discussion
Our data demonstrate for the first time that syndecan-1 belongs to epithelial podosomes and that its expression within their outward ring is required for their formation and the subsequent MMP-9 activity. Our data further reveal that the laminin LG45 domains increase their number and MMP-9 activity in a manner comparable to that of IL1β. Syndecan-1 distribution in filopodia at the front edge of migrating keratinocyte may have a role to play in the regulation of MMPs activity therefore facilitating their path to regenerate the epidermal compartment.

P75, Characterization of inflammatory breast cancer: a vibrational microspectroscopy and imaging approach at cellular and tissue level

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Introduction

Inflammatory breast cancer (IBC) has a poor prognosis because of the lack of specific biomarkers and late diagnosis. An accurate and rapid diagnosis implemented early enough can significantly improve disease outcome. Vibrational spectroscopy has proven useful for cell and tissue characterisation based on intrinsic molecular information. Here, we have applied infrared and Raman microspectroscopy and imaging to differentiate between non-IBC and IBC at both cell and tissue levels.

Materials and Methods

Two human breast cancer cell lines (MDA-MB-231 and SUM-149), 20 breast cancer patient (10 non-IBC and 10 IBC), and 4 healthy volunteers biopsies were investigated. Fixed cells and tissues were analyzed by FT-IR microspectroscopy and imaging, while live cells were studied with Raman microspectroscopy. Spectral data were analyzed by hierarchical cluster analysis (HCA) and common K-Means algorithms.

Results

For both cell suspensions and single cells, FT-IR spectroscopy showed sufficient high inter-group variability to delineate MDA-MB-231 and SUM-149 cell lines. Most significant differences were observed in the spectral regions of 1096-1108 and 1672-1692 cm^{-1}. Analysis of live cells by Raman microspectroscopy gave also a good discrimination of these cell types. The most discriminant regions were 688-992, 1019-1114, 1217-1375 and 1516-1625 cm^{-1}. Finally, K-Means Cluster analysis of FT-IR images allowed to delineate non-IBC from IBC tissues.

Discussion

This study demonstrates the potential of vibrational spectroscopy and imaging to discriminate, without dewaxing, between non-IBC and IBC at both cell and tissue levels. A new concept based on spectral histology identifies non-IBC and IBC tissues in a label-free manner showing promises for diagnosis of breast cancer.

P76, Effects of hypoxia on extracellular matrix synthesis by human coronary artery endothelial cells

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Introduction
As a multifactorial chronic inflammatory disease, there are many influences on both the initial formation and development of advanced atherosclerosis and this can lead to plaque rupture, and consequently heart attack and stroke. Increased metabolic demand for oxygen and gas diffusion distance from arterial lumen lead to localised hypoxia (<1% O2) is a known feature of atherosclerosis. This study aims to investigate the effects of hypoxia in altering the composition of the vascular extracellular matrix (ECM), another hallmark of atherosclerosis.

Materials and Methods
Human coronary artery endothelial cells (HCAECs) were cultured under either hypoxic (1% O_2) or normoxic (20% O_2) conditions. Gene expression of ECM, hypoxia-induced cytokines and cell activation markers were investigated via real time qPCR, while presence of ECM proteins was detected using ELISA and Western blotting. Cell adhesion and proliferation assays of HCAECs under hypoxic condition were also carried out using Calcein AM and MTS reagent respectively. Cell activation markers ICAM-1, VCAM-1 expression and reactive oxygen species (ROS) production were also studied by flow cytometry.

Results
After 24 hours exposure to hypoxia, HCAECs showed increased mRNA expression of HIF1a, VEGF and VEGFR. There was no significant increase in ICAM-1 gene expression (qPCR) and protein expression (flow cytometry) of HCAECs in response to 7 days of hypoxia exposure, however, increased ROS production was detected in HCAECs exposed to hypoxia. Gene expression of a panel of ECM components was investigated, expression of laminin α1 and versican increased significantly in HCAECs exposed to hypoxia compared to normoxic control group. Increased expression of versican protein was also mirrored in ELISA. Interestingly, loss of HCAEC adhesion was observed in response to hypoxia but adhered HCAEC was more proliferative when cultured under hypoxic condition.

Discussion
These results suggest that an altered ECM is produced in response to hypoxia, and hypoxia may play a role in the progression in chronic inflammatory diseases such as atherosclerosis. We are going to continue to investigate the splice variants of versican by both qPCR and Western blot. Future study will also be done on mechanisms underlying hypoxia induced cellular behavioural changes, and synthesis of matrix metalloproteinases which are known to play a role in remodelling the ECM components in diseased sites.

P77, A *Col9a3* EXON 3 SKIPPING MOUSE AS NOVEL MODEL FOR MULTIPLE EPIPHYSEAL DYSPLASIA

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Introduction
Multiple epiphyseal dysplasia (MED) is an autosomal chondrodysplasia characterized by early-onset degenerative joint disease and epiphyseal hypoplasia. Its genetic background is complex and heterogeneous since mutations in several genes coding for extracellular matrix (ECM) components have been identified in patients. Among these genes are those encoding for the pro-α chains of the Type IX collagen, COL9A1, COL9A2 and COL9A3 where the majority of mutations lead to the skipping of exon 3.

Materials and Methods
By CRISPR/Cas9 technology we generated a mouse carrying a deletion of *Col9a3* exon 3 (*Col9a3*$^{\Delta ex3}$), reproducing the splicing events reported in a MED patients group. Initial phenotyping of 3- and 9-week old *Col9a3*$^{\Delta ex3}$ mice is underway including skeletal X-ray and growth plate analysis (through immunohistochemistry and BrdU labelling to monitor chondrocyte proliferation).

Results
CRISPR/Cas9 injection generated mice with the differing genomic deletion of exon 3, the breakpoints of which were confirmed by Sanger sequencing. The phenotyping of offspring through DNA and cartilage RNA analysis had led to the establishment of two transgenic mouse lines, one splicing as predicted (*Col9a3*$^{\Delta ex3}$) and a second almost completely lacking the Col9a3 transcript (*Col9a3*$^{-/-}$). Immunoblotting confirmed the lack of Collagen type IX protein from predicted null mouse cartilage. Both lines are viable, however only *Col9a3*$^{-/-}$ mice displayed detectable phenotypic abnormalities: mild short stature and hip dysplasia, abnormal tibial epiphysis morphology and reduced level of chondrocyte proliferation in a disorganized growth plate structure. No overt phenotype detected instead in *Col9a3*$^{\Delta ex3}$ line.

Discussion
Having confirmed the production of a shorter RNA from cartilage of *Col9a3*$^{\Delta ex3}$ mice, lacking only exon 3 and therefore splicing as expected and producing Collagen type IX protein, no overt phenotype was detected, therefore the future work will be focused on assessing their cartilage stability. *Col9a3*$^{-/-}$ line instead, has a mild skeletal phenotype and shows no transcript and protein, results confirmed by WB and immunohistochemistry of the growth plate.
Both mutant mice will represent an important tool to gain insights on Collagen IX structure and its role into the matrix. In particular, the exon skipping line, by recapitulating human Col9-MED, can add to our understanding of the disease mechanism responsible for the onset of MED.

P78, Overexpression of Fibulin-7 modulates the Ang1-Tie2 system and contributes to the aberrant vasculature in glioblastoma

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Introduction
Glioblastoma multiforme (GBM) is characterized by high invasiveness and hypervascularity, the latter of which is distinct from normal vasculature, showing hypertrophied and glomeruloid vessels. This complex vasculature involves an active remodeling of the extracellular matrix (ECM) by production of new ECM molecules and degradation of the existing ones. Fibulin-7 (Fbln7), an ECM protein of the Fibulin family, has recently been reported to be overexpressed in brain tumors by Cancer Microarray Databases. In this study, we analyzed its expression and role in gliomas.

Materials and Methods
Astrocytic tumor and normal brain tissue samples were obtained from Juntendo and Kanazawa University Hospitals (Japan). Immunoblotting, immunohistochemistry and confocal microscopy were used to visualize the protein expression. Solid-phase binding and pull-down assays were performed to assess the protein-protein interaction. We developed a new endothelial cells-pericytes co-culture assay to mimic the aberrant vasculature *in vitro*.

Results
Tissue immunoblotting analyses showed that Fbln7 is expressed in gliomas and appeared to be overproduced in a grade-dependent manner. Quantification analysis demonstrated the highest expression in GBM compared with the control normal brain or the lower-grade astrocytomas. Immunohistochemically, Fbln7 was highly expressed by endothelial cells and pericytes in the aberrant vessels besides a lower expression by glioblastoma cells. Fbln7 specifically bound to Ang1, but not Ang2 or Tie2, and inhibited the Ang1-Tie2 signal in cultured endothelial cells. In an endothelial cells-pericytes co-culture system, high concentration of VEGF up-regulated Fbln7 expression and resulted in formation of aberrant vessel-like structures, which were suppressed by treatment with neutralizing anti-Fbln7 antibody, Fbln7 peptides or siRNA-mediated down-regulation of Fbln7.

Discussion
We showed the overexpression of Fbln7 in the endothelial cells and pericytes of the aberrant microvascular vessels in GBM. Our study suggests that Fbln7 overproduced by these vascular cells is involved in the formation of dysmorphic blood vessels in GBM through modulation of the Ang1/Ang2-Tie2 signaling pathways by interacting with Ang1. GBM is characterized by an abnormal vasculature, which is one of the molecular targeting therapies. Our data suggest that suppression of Fbln7 function by antibodies or peptibodies or down-regulation of Fbln7 expression may be an attractive target for therapies of GBM when combined with other anti-angiogenic drugs.

P79, Proteomic screening identifies the zonula occludens protein ZO-1 as a new partner for ADAM12 in invadopodia-like structures.

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Introduction
The epithelial mesenchymal transition (EMT) is a key process for cancer cell invasion and migration. This complex program whereby epithelial tumor cells loose polarity and acquire mesenchymal phenotype is driven by the regulation of cell-cell adhesion and cell-substrate interactions. Recently, we demonstrated that the long form of ADAM12 protein promotes EMT. In the present study, we identify ZO-1 as a new partner for ADAM12 during this process and we investigate the role of this complex in invasiveness.

Materials and Methods
To identify interacting partners for ADAM12 we used immunoprecipitation and proteomic approaches on ADAM12L-overexpressed MCF10A cells. By in silico screening we searched for breast cell line that expressed endogenous ADAM12 and ZO-1. We validated the interaction by immunoprecipitation, by proximity ligation assay and by immunolocalization. By siRNA of ADAM12 or ZO-1 we demonstrated their role in matrix degradation and invasion.

Results & Discussions:
A proteomic approach allowed to identify ZO-1 as new partner of ADAM12 during EMT. We showed that ZO-1 and ADAM12 were co-expressed in invasive breast cancer cell lines sharing EMT gene signatures. We validated the interaction between ZO-1 and ADAM12L in breast cancer invasive cell lines where they colocalized in invadopodia-like structures together with membrane type 1 matrix metalloprotease (MT1-MMP) whose activity is required for matrix degradation. In addition, silencing ADAM12L or ZO-1 expression inhibits the activity of matrix degradation and the invasiveness of these cells. Interestingly, we observed that silencing ADAM12L disrupts the localization of ZO-1 at the level of invadopodia-like structures demonstrating the role of ADAM12L in the translocation of ZO-1 to these structures. This distribution of ADAM12 and ZO-1 in the invadopodia type structures is dependent on PKCε protein whose invalidation blocks not only the localization of these proteins but also the activity of matrix degradation and invasion. Together our data provide evidence for a new interaction between ADAM12, a mesenchymal marker and ZO-1, a scaffolding protein expressed in tight junctions of epithelial cells, both proteins being redistributed at the invadopodia-like structures to promote PKCε-dependent matrix degradation.

P80, Hyaluronan synthesis in fibroblasts is induced by a novel cancer secreted factor

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Introduction
Interaction between cancer cells and their microenvironment is central in defining the fate of cancer development. Tumour cells secrete specific signals that modify the surrounding area and the niche supplies structures and activity necessary to survival, growth and development of the cancer cells. In particular, extracellular matrix molecules, like the glycosaminoglycan hyaluronan (HA), are known to influence tumour cell functions, such as proliferation, migration and neoangiogenesis. Here we show that a yet uncharacterized soluble factor, named c10orf118, secreted by breast tumour cell lines, can induce the secretion of HA by stromal fibroblasts through the up-regulation of the hyaluronan synthase 2 gene (*HAS2*).

Materials and Methods
Conditioned medium from 9701-BC cells was loaded on SDS-PAGE and the representative proteins analysed by MALDI-TOF. The protein expression was investigated in other breast tumour cell lines (MCF-7 and MDA-MB231) by RT-PCR analysis. Co-culture of MCF-7 and normal human dermal fibroblasts was performed to check the rate of expression of *HAS2* and the secretion of HA by fibroblasts.

Results
MALDI-TOF analysis of a band in SDS-PAGE from conditioned medium by 8701-BC cells, showed peptides that matched for the c10orf118 protein with the accession number NM_018017 in NCBI/BLAST, corresponding to Q7z3E2 according to the Uniprot identifier. This protein is also expressed by MCF-7 and MDA-MB231 cells, both at intracellular level and secreted in the medium. When co-cultured with MCF-7, fibroblasts showed enhanced expression of *HAS2* and a corresponding increment in HA secretion. These effects could be abolished when the MCF-7 conditioned medium was pre-incubated with an anti-c10orf118 antibody.

Discussion
In this study we have identified a novel soluble agent that modulates the HA secretion by stromal cells and therefore takes part in tumour matrix remodelling
The expression of the c10orf118 protein associates in cancer patient specimens with the presence of estrogen receptor α, whose signaling pathway has been shown to be influenced by *HAS2* overexpression. We suggest c10orf118 as a new player in regulating the microenvironment remodeling of breast cancer cells, possibly influencing the aggressiveness of the cancer.

P81, LaNt α31, a novel regulator of basement membrane assembly

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Introduction
The laminin N-terminus (LaNt) proteins are a family of laminin and netrin-related proteins that are derived by alternative splicing from laminin encoding genes. To date, there is only one report of LaNt function, with one family member, LaNt α31, shown to regulate keratinocyte migration and adhesion through an as yet uncharacterised mechanism. Based on protein architecture, we predicted that LaNt α31 influences laminin organisation.

Materials and Methods
Adenoviral-mediated overexpression of LaNt α31 with C-terminal GFP tag was induced in corneal and epidermal keratinocytes. The impact on cell behaviours was assessed using live cell imaging. LaNt α31 protein interactions and their influence on matrix assembly were determined using immunoprecipitation, indirect immunofluorescence, total internal reflection and live confocal microscopy.

Results
LaNt α31 co-distributed and co-immunoprecipitated with laminin β3 in the extracellular matrix, while live cell assays revealed that the proteins are deposited together during new matrix synthesis. Moreover, induced expression of LaNt α31 led to changes in laminin α3 organisation, forming tight clusters in contrast to the typical broad arcs in control cells. These changes were associated with premature maturation of hemidesmosomes and mis-localisation of focal adhesion complexes. Epithelial cells expressing LaNt α31 GFP also displayed decreased scratch closure and single cell motility rates, and increased cell spreading. All of these aspects could be rescued through provision of a pre-formed matrix indicating a matrix deposition affect rather than cellular defect.

Discussion
Together these data identify a new protein that can influence the early stages of laminin matrix assembly, introducing the LaNts as new players in regulating cell adhesion and migration.

P82, *Lgr5* and *Col22a1* mark progenitor cells in the differentiation toward juvenile articular chondrocytes

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Introduction
Skeletal movement is facilitated by synovial joints comprised of articular cartilage encased in a capsule. Articular cartilage wears with aging and repair is inefficient, and reason not well understood. In general, tissue repair involves the recruitment and/or activation of local progenitor cells, initiating cellular differentiation similar to embryonic development for repair. Synovial joints are formed from a pool of progenitor cells in the interzone which is a temporary structure during embryogenesis marked by the expression of Gdf5. However, the differentiation and lineage specification toward the different structures of the joint is not well studied. We identified *Lgr5* as a novel marker for interzone cells from an analysis of stem cell related genes.

Materials and Methods
We characterized the expression of *Lgr5* and traced *Lgr5*-descendent cells in the limb joints in development and early postnatal growth with Lgr5-GFP-CreERT mice. We collected Lgr5-GFP expressing (Lgr5+) cells in the interzone by FACS for transcriptomic analysis using RNA-sequenicng to gain insights into the molecular signature and potential ECM niche of these cells.

Results
We have identified a population of Lgr5+ interzone cells in the central region of the interzone that contribute to the formation of cruciate ligaments, synovial membrane and articular chondrocytes of the joint. From the transcriptome data, we identified ECM genes that are enriched in the interzone comparing to the surrounding chondrocytes that included Collagen XXII (*Col22a1*). We showed *Col22a1*, a marker of early articular chondrocytes, co-expresses with Lgr5 in progenitor cells along the lineage differentiation towards articular chondrocytes.

Discussion
We identified novel gene markers along the progressive differentiation of progenitor cells to articular chondrocytes. A clear understanding of these progenitor cells in joint formation and their niche will provide insights into the molecular controls with the prospect of using these cells for effective joint repair.

P83, Intracellular activities for an extracellular protein - Lysyl oxidase intracellular activity regulates VGLL3 nuclear localization in satellite cells

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Introduction
One of the most prominent pathological features of muscular diseases is the fibrotic reaction. A key fibrotic player upregulated in multiple diseased muscles is Lysyl oxidase (LOX), an enzyme best known for its extracellular matrix crosslinking activities.

Methods and Results
To better dissect its roles, we generated mice that over-express LOX and monitored the effects on muscle regeneration. Surprisingly, we find that in these mice no apparent fibrosis is observed, yet they regenerate faster than their wild-type littermates suggesting LOX acts in other, novel mechanisms during myogenesis. To dissect these activities, we inhibited its enzymatic activity following muscle injury and examined the resulting effects on muscle regeneration. We find a reciprocal response to that observed in the LOX-over expressing mice where termination of the regeneration process is inhibited, resulting in immature myofibers and satellite cells maintained in an active state. To identify the underlying mechanism, we generated a *Lox* conditional knockout allele that is responsive to Cre activation. Unexpectedly, we find that although Lox is upregulated by multiple cells within the regenerating muscle, its deletion only in satellite cells using *Pax7-Cre^{ERT}*, attenuates muscle regeneration similarly to that following its pan inhibition. Cell culture assays, demonstrate LOX is acting autonomously in balancing myoblast proliferation vs. differentiation. A screen for LOX binding partners identified Vestigial Like 3 (VGLL3), a transcriptional co-activator acting with multiple DNA-binding partners within the muscle, including MEF2 and TEAD proteins, to specifically interact with LOX, an interaction which is dependent on Lox enzymatic activity.

Discussion
Our results suggest that LOX enzymatic activity regulates VGLL3 nuclear localization. Altogether, our results demonstrate a novel, hitherto unidentified intracellular role for LOX in regulating the transcriptional output crucial for the execution of myogenic differentiation and muscle regeneration.

P84, Dermal collagen XII has a dose-dependent effect on skin repair

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The FACIT collagen XII is expressed in stiff connective tissues where it decorates larger collagen fibrils. Mutations or loss of collagen XII cause disorders of connective tissues in humans, e.g. hyperelastic skin, indicating a vital role of collagen XII in development and ECM homeostasis. We postulate that collagen XII has the potential to influence tissue properties by interconnecting structural molecules, transmitting matrix-derived signals to cells or by modulating bioavailability of growth factors.
We aim to study the roles of dermal collagen XII during reconstitution of the dermis following excisional wounding in wild type and in mouse lines with fibroblast-specific overexpression or systemic deletion of collagen XII.
In wild type mice, we observed an increased deposition of collagen XII into the early granulation tissue after injury that persisted at high levels throughout the healing process. The knockout and the overexpressing mouse models both showed delayed wound healing, but a different cellular composition in the granulation tissue. Loss of collagen XII caused wider wounds with elevated numbers of macrophages and myofibroblasts and increased TGFβ signalling. By contrast, overabundance of collagen XII led to thicker wounds with a higher overall cell density and macrophage numbers but without impact on myofibroblasts. As both conditions caused increased macrophage numbers we further characterized the interaction between macrophages and collagen XII *in vitro* and found that only M1 polarized macrophages were able to adhere to collagen XII, but not unstimulated and M2 polarized macrophages.
These findings led us to the hypothesis that lack of collagen XII causes a paucity of M1 polarized macrophages and a relative increase in M2 polarized pro-fibrotic macrophages that secrete TGFβ and stimulate myofibroblast differentiation. On the other hand, the overproduction of collagen XII favours a selective adhesion of M1 polarized pro-inflammatory macrophages. To test our hypothesis that collagen XII can control the healing process and possibly its outcome by regulating macrophage populations we will characterize macrophages within the granulation tissue of the two mouse models in detail and investigate the receptor reservoir that mediates the selective adhesion of M1 polarized macrophages to collagen XII.

P85, *Wisteria floribunda* agglutinin, a perineuronal net marker, demonstrates differential binding affinities to various chondroitin sulphates

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Introduction
Perineuronal nets (PNNs) are a condensed form of extracellular matrix that encase neurons in the central nervous system and are implicated in a number of disease pathologies and potential treatments; including schizophrenia, epilepsy, autism; spinal cord injury, Alzheimer's disease, addiction and stroke. PNNs contain chondroitin sulphate glycosaminoglycans (CS-GAGs) of which there are 5 subtypes; CS-A to CS-E.
Wisteria floribunda agglutinin (WFA) is a plant lectin which is considered the main marker of PNNs and binds to CS-GAGs in the nets. It is currently thought to specifically bind the GalNAc moiety of the CS-GAGs, if this is the case then WFA should bind all PNNs. However evidence has shown that not all PNNs are bound by WFA, thus we hypothesised that WFA does not simply bind GalNAc but may be specific to certain subtypes of CS-GAG.

Materials and Methods
Quartz crystal microbalance with dissipation monitoring was used to interrogate the PNN/CS interaction. CS-GAG chains of each subtype were secured to an oscillating surface. Mass adsorbed to the surface, in the form of molecules binding, could be detected via a decrease in the frequency of its oscillation.

Results
After addition of WFA at 100 µg/mL to; CS-B (DS), CS-D, CS-E there were frequency shifts of -8Hz, -5Hz and -49Hz respectively. This suggests that WFA binds CS-E with the highest affinity and can also bind CS-B (DS) and CS-D with lower affinity. CS-A and CS-C showed no frequency shift above that of the control (control: no CS chains bound to surface) indicating that WFA does not bind CS-A and CS-C.

Discussion
The results indicated that WFA is more specific than previously reported, which should be taken into consideration when it is used as a PNN marker. Use of an additional PNN marker such as aggrecan may be advised.

P86, Molecular basis of Asporin as a genetic risk factor for intervertebral disc degeneration

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Introduction
Intervertebral disc degeneration (IDD) is a major cause of back pain, affecting quality of life as we age. While the environment, lifestyle and aging contribute; genetic has a major effect on susceptibility, onset and severity. Previously, we identified Asporin (ASPN), an extracellular matrix (ECM) protein in the intervertebral disc as a risk factor. In addition, increased *ASPN* expression is associated with IDD. *ASPN* appears to have roles in the induction and progression of IDD, but the molecular and degenerative mechanism is not understood.

Materials and Methods
ASPN-tg mice were generated, over-expressing human ASPN under the control of the mouse *Col9a2* promoter, allowing expression in the cartilage endplate and nucleus pulposus (NP) of the intervertebral disc (IVD), leading to changes similar to human IDD. Molecular and cellular changes in the IVD were analysed to gain functional insights. We analysed cell morphology and gene markers for chondrocyte and NP cell maintenance, and performed a proteomic analysis of the NP tissue to assess global and specific changes.

Results
We identified enhanced TGF-β signalling in the NP of ASPN-tg mice, resulting in accelerated chondrogenic events. Interestingly, the most significant change in intervertebral disc (IVD) proteome of ASPN-tg mice is a reduced detectable level of Fibrillin-1 (*FBN1*), a micro-fibril forming ECM protein that limits TGF-β activation by sequestering the large TGF-β latent complex. Further, changing the genetic background, from C57B/6 to LG/J, considered being poor and good “healer” of damaged tissues respectively, can ameliorate the risk imposed by ASPN on IDD.

Discussion
ASPN-tg mice provided novel mechanistic insights into the functional role of ASPN in the maintenance of NP tissue integrity and function; a process likely to be related to a fine-tuning of TGF-β signalling for ECM integrity, such as the level of FBN1. Indeed, genetic mutations in *FBN1* are associated with the Marfan syndrome, resulting in excessive TGF-β signaling. ASPN-tg mice will provide a mean for additional studies to assess TGF-β signalling and potential of therapeutic interventions, and the possibility to perform genetic mapping to identify protective factors for IDD in the LG/J genetic background.

P87, PCSK9 influence the hyaluronan and proteoglycan metabolism in vascular cells

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Introduction
Proprotein convertase subtilisin/kexin type 9 (PCSK9) is involved in regulation of plasma low-density lipoprotein cholesterol (LDL-C) levels in blood directly binding LDL receptor (LDLR) and enhancing its lysosomal degradation in hepatic cells. Therefore, PCSK9 is considered as a major drug target in cardiovascular disease, and antagonizing plasma PCSK9 causes remarkable reductions in LDL-C levels. A new line of investigation has emerged including effects of PCSK9 on matrix biosynthesis in vascular cells. Recently, our lab addressed the biology of the atherosclerosis outcome by using vascular cells treated with LDL and oxidized LDL. Such process leads to local responses ending in the production of atherosclerotic plaques. In the early stages of atherosclerosis, hyaluronan (HA) production by endothelial and smooth muscle cells (SMCs) is critical to form the endothelial glycocalyx that protects vascular tissues from inflammatory cells activation and SMCs are essential in neointima formation. The aim of our study to explore the effect of PCSK9 in the matrix molecules metabolism in endothelial and smooth muscle cells.

Materials and Methods
Endothelial cells (HUVEC) and smooth muscle cells (hAoSMC) were treated with 20 ug/ml of LDL (normal, oxidized or cholesterol depleted) and/or PCSK9 (80 or 100 ng/ml) for 24 hours. Several genes expression were quantified using real-time PCR.

Results
We demonstrated that inflammatory stimuli are able to increase the synthesis of HAS2 and not HAS3 in vascular cells and modulate the syndecan 1 and 4 expression on endothelial cells. The incubation of HUVEC cells with PCSK9 partially reduced the LDLR and the receptor for transcytosis ALK1, which are the most expressed in HUVEC. More interestingly, the scavenger receptor LOX1 was dramatically reduced. Preliminary data indicate that the treatment with PCSK9 alters HA and CD44 synthesis both in HUVEC and SMC. In order to evaluate the direct effect of cholesterol on the cells, using cholesterol depleted LDL, we found an alteration of HA synthesis with these cholesterol free LDL. These data indicate that HA synthesis depends on the cholesterol amount in LDL and therefore inside the cells.

Discussion
These data support the physiological role played by PCSK9 in HA metabolism by influencing cholesterol content in the cells.

P88, Intracellular trafficking of the invasion promoting cell surface proteinase MT1-MMP

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Introduction
Membrane type-1 matrix metalloproteinase (MT1-MMP) is a transmembrane proteinase, which has been shown to promote progression of several diseases including rheumatoid arthritis and cancer by enhancing cellular invasion. MT1-MMP promotes cellular invasion by degrading pericellular extracellular matrix (ECM), thus its localisation to the leading edge of the cell is crucial. However, the mechanism has not been clearly understood. We have found that MT1-MMP cell-surface exposure is achieved by intracellular trafficking of MT1-MMP containing vesicles along microtubules and identified four kinesin motor superfamily proteins (KIFs) involved in this process.

Materials and Methods
HT1080 human fibrosarcoma cells transfected with siRNA targeting KIFs were subjected to functional analysis of MT1-MMP, including gelatin film degradation assay and collagen film degradation assay. Cell surface localisation of MT1-MMP was analysed by confocal and total internal reflection fluorescence (TIRF) microscopies.

Results
Our data show that the knockdown of four KIFs affects MT1-MMP activity on the cell surface: silencing some KIFs decreased MT1-MMP proteinase activity against gelatin and collagen film, while the knockdown of other KIF enhanced MT1-MMP activity. Interestingly, silencing these KIFs does not affect the overall level of MT1-MMP on the cell surface, while it significantly influences MT1-MMP localisation at the substrate-attached sites of the cells.

Discussion
These data suggest that these four KIFs play key roles in regulating MT1-MMP cell-surface localisation. We hypothesize that each of the four KIFs plays different roles in localising MT1-MMP to the substrate-attachment site. Further investigation of MT1-MMP vesicle transport using live cell imaging techniques will allow us to understand dynamics regulation of MT1-MMP localisation to promote cellular invasion.

P89, Contradictory effects of electroporation on human cutaneous cell migration and proliferation in perspective of wound healing

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Introduction
When the cell is exposed to high external electric field the plasma membrane becomes permeable to ions, drugs and molecules as large as plasmid DNA, which are otherwise impermeable: this is electroporation phenomenon. A medical application of electroporation is gene electrotransfer, which is highly promising in wound healing by transferring relevant therapeutic plasmid. Interestingly, in this context, some authors demonstrated that electric field applied alone significantly improved wound closure. Besides, the main clinical application of electroporation in medicine is electrochemotherapy. In this case also, clinicians observed aesthetic and functional wound healing of the treated sites. In brief, even if cellular mechanisms are not yet deciphered, there is a strong link between wound healing and electroporation.

Materials and Methods
We determined the effects of these electric field parameters on primary dermal fibroblasts and keratinocytes isolated from human skin biopsy. We also worked, as a control, with a human melanoma cell line A375.

Results
Gene electrotransfer electric parameters (10 pulses lasting 5ms at 1Hz with intensity from 50 to 300 V/cm) did not induced any cell modification in terms of proliferation and migration abilities for the three cutaneous cell types. Contrariwise electrochemotherapy electric parameters (8 pulses lasting 100µs at 1Hz with intensity from 200 to 800V/cm) exerted three distinct effects on the cutaneous cell types. While migration of fibroblasts was stimulated, keratinocytes migration was inhibited, probably because of cell fusion. For A375 melanoma cells, no effect was observed in terms of migration ability.

Conclusion
Further studies are needed to deepen the comprehension of underlying cell mechanisms activated by cell exposition to electroporation. A special focus will be held on extracellular matrix production by fibroblasts after electrostimulation.

Discussion
It is known that a cutaneous wound creates a break in the epithelium and its transepithelial potential which generates an endogenous electric field at the wound margins. In healthy context this endogenous electric field stimulate surrounding cell to achieve an optimal healing, but in chronic wounds, this endogenous electric field is lost. Albeit far from this endogenous electric field characteristics, it seems that electric fields used in electroporation present a potential for electro-stimulation of cutaneous cells in a perspective of wound healing.

P90, Heterogeneity of Cancer Associated Fibroblasts: Metastatic potential and time determine CAF population composition

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Introduction
The metastatic process is heavily influenced by the tumour microenvironment. Cancer-associated fibroblasts (CAFs) are a major class of stromal cells with many, sometimes seemingly conflicting, functions reported. Importantly, CAFs are not a homogenous population of cells. Studies have shown the presence of CAF subpopulations with varying expression patterns of markers, and no specific marker has yet been found to identify all fibroblasts within a tumour. Despite this, CAF heterogeneity is often overlooked in functional studies with only one or two markers being used as identifiers. This approach leads to under-sampling of the entire CAF population and important functional information remains unresolved. We aim to understand how CAF marker heterogeneity corresponds to functional differences. Deciphering the pro- and anti-tumorigenic potentials of CAF subpopulations will deepen our understanding of CAF complexity and ultimately help guide targeted therapies to the correct sub-population(s).

Materials and Methods
Orthotopic, syngeneic murine breast cancer tumours of different ages and metastatic potential were analysed through multi-colour flow cytometry. After lineage exclusion of cancer cells and non-fibroblast stromal cells, the CAF-enriched, lineage- population was analysed for combinatorial expression of 6 fibroblast markers.

Results
The composition of the CAF-subpopulations within aggressively metastatic tumours was substantially altered from nascent to well-established tumours. However, in less aggressive tumours this change was less pronounced and also different from that of aggressive tumours. Additionally, none of the chosen markers were pan-CAF markers, and importantly, even the combination of all 6 markers did not capture all of the lineage- CAF-population.

Discussion
We here identify how a set of 6 commonly used fibroblast markers outline distinct sub-populations of CAFs, and that the composition of CAF sub-populations changes as tumours mature, indicating co-evolution of the CAF population with the tumour. Combining all 6 fibroblast markers failed to label all of the lineage- CAFs, underscoring the heterogeneity of CAFs, and the need for novel CAF markers.

P91, Collagen XIII contributes to transsynaptic adhesion in the developing neuromuscular junction

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Introduction
Collagen XIII is a type II transmembrane protein associated with neuromuscular and myotendinous junctions in the muscle. In the neuromuscular junction, collagen XIII localizes to the synaptic basal lamina and postsynaptic membrane. Loss-of-function mutations in the *COL13A1* gene lead to congenital myasthenic syndrome type 19. Typical symptoms are muscle weakness and fatigability, leading to respiratory and feeding difficulties and thus the condition is usually very severe. Collagen XIII has been found necessary for correct morphology, transsynaptic adhesion, alignment, maturation and function of the neuromuscular junction, but little is known about its effects during embryonic neuromuscular development.

Materials and Methods
The diaphragm was collected as a whole-mount preparation during embryonic development from wild-type and collagen XIII-knockout mouse foetuses and stained for neuromuscular junctions and motor neurons. Samples were analysed with confocal microscopy followed by quantification and statistical analyses.

Results
In mouse foetuses lacking collagen XIII, the pattern formed by neuromuscular junctions is significantly wider in the diaphragm muscle at embryonic day 16,5. Also, in the absence of collagen XIII there is an increased number of motor neuron axons that fail to stop at the neuromuscular junctions, growing beyond them instead. Preliminary results indicate that lack of collagen XIII leads to increased branching of the phrenic nerve and especially the number of terminal branches appears larger.

Discussion
These findings suggest that collagen XIII has a significant role in transsynaptic adhesion between muscle and nerve, and in conveying an attachment signal to establish the motor synapse during neuromuscular development. In addition, collagen XIII expression has been detected in vasculature surrounding the phrenic nerve and other peripheral nerves, and the increased branching of the phrenic nerve observed in collagen XIII-knockout mice might suggest a role for collagen XIII in regulating axon outgrowth.

P92, Validation of murine primers for the characterization of Heparan Sulfate biosynthesis enzymes during physio-pathological processes

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Introduction
Heparan sulfate (HS) is a family of linear sulfated glycosaminoglycans linked to core protein to form proteoglycans, on cell membrane or in the extracellular matrix. HS are characterized by D-Glucuronic Acid / N-Acetyl-D-Glucosamine disaccharides polymerized in chains and modified by several classes of enzymes, like sulfotransferases (OSTs) that add sulfates at various positions of the disaccharides. HS structures and functions are modified during pathophysiological evolution of a tissue, such as aging or injury, then it's very important to study pattern of expression of all the enzymes involved in HS biosynthesis.

Materials and Methods
We present molecular biology tools and reliable quality criteria, such as primers sequences design and tissue controls validation, for specific RT-qPCR analysis of the expression pattern of 26 enzymes linked to HS modification in murine models. RT-PCR analysis were performed according to Agilent Technologies, to determined Cq, Standard and Melting curves to validate amplicons purity and Tm dissociation.

Results
All primer pairs must respect several design criteria in order to be specific of the gene of interest. This specificity as well as the amplification efficiency were validated in tissues positive controls identified *in-silico* as putative on https://www.proteinatlas.org/ database. Most of the enzymes are expressed in neural tissues, such as Cortex and Cerebellum, as well as in spleen, rate and chondrocytes, that were used as positive control. Finally, all enzymes expressions were analyzed in 2 models of Osteoarthritis, a degenerative pathology of the cartilage characterized by a strong remodelling of the glycanic compounds of the matrix : (1) *in-vitro* on primary culture of murine chondrocytes induced toward hypertrophic phenotype by IL-1 and (2) *in-vivo* in mice with chirurgical Destabilization Medial Meniscus.

Discussion
The molecular tools developed here permit to complete our glycomic platform : data on structural evolution of GAG that we recently obtained in OA can be now correlated to HS enzymes expression patterns. This confirms our ability to identify glycanic targets for new therapeutic strategy of degenerative pathologies.

P93, Fibroblast state switching orchestrates dermal maturation and wound healing

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Introduction

The maturation of dermis, as well as wound healing, is a tightly controlled combination of fibroblast proliferation, quiescence, and extracellular matrix (ECM) secretion and organization. Here we explore the transitions in fibroblast behaviour by developing a novel virtual tissue model for recapitulating how fibroblast subpopulations emerge, arrange and influence each other during development and wound healing.

Materials and Methods

The methods combine state-of-the-art mathematical and computational modelling, *in vivo* live imaging, histological analysis (including collagen analysis by picrosirius, Collagen Hybridizing Peptide, and TEM), lineage tracing, as well as *in vitro* experiments (collagen gel and organotypic culture) with isolated human and mouse fibroblasts.

Results

Based on the *in vivo* and *in silico* analysis of fibroblast behaviour, we propose a model where dermal architecture is determined by a negative feedback loop between ECM deposition and proliferation. *In vitro* experiments with primary fibroblasts supported the idea of ECM-proliferation feedback loop and showed that ECM deposition alone is sufficient to drive neonatal fibroblast to quiescent state. On the contrary, the destruction of collagen mimicking the wound situation activated the proliferation of fibroblasts. Our model describes dermal maturation process and the loss of spatial segregation of fibroblast lineages. In addition, the model suggests that migration of fibroblasts is needed for wound healing but not for developmental maturation leading to the homeostatic skin architecture. These predictions were confirmed by *in vivo* analysis.

Discussion

Our results provide a mechanistic insight how the fibroblasts behaviour is coordinated to reach the normal maturation of dermis and wound healing. This will be useful for understanding the pathological processes of skin and for identifying new potential therapies.

P94, Endothelial cell-derived MMP-14 is dispensable for skin formation and repair

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Angiogenesis, the formation of new blood vessels from pre-existing ones, is a natural and crucial process during development and in adulthood. MMP-14, a zinc-dependent endopeptidase, plays a critical role in angiogenic processes. MMP-14 deficiency is lethal within 3 weeks and mice display severe skeletal defects. To investigate the cell-autonomous role of MMP-14 expression in endothelial cells, we generated a conditional knockout mouse line with endothelial cell-specific depletion (Tie2-driven Cre) of MMP-14 (MMP-14 EC-/-). These mice are mostly normal and have a normal lifespan. Similar to the complete MMP-14 knockout the skull of MMP-14 EC-/- mice at postnatal day 7 displayed slightly delayed suture closure, a slightly domed skull, and a shortened snout previously accredited to the defective osteoblast-MMP-14 activities. Strikingly our data demonstrate that this phenotype can result from ablation of MMP-14 in endothelial cells, revealing a crucial role for endothelial MMP-14 in membranous ossification and suture closure. Adult mice (3 months) also developed shortened snouts with deviations of the midface whereas long bones (femurs) were completely normal.
Histological skin analysis revealed overall normal skin morphology and epidermal differentiation patterns. Also wound repair in MMP-14 EC-/-, including kinetics, re-epithelialization, epidermal differentiation and granulation tissue formation were comparable with wild-type mice. Further, no altered neovascularization at day 5 and day 17 was shown (CD31/SMA stainings), and collagen density and fibers at later time point were comparable to control mice. To investigate whether single MMP-14 deficient endothelial cells showed molecular and functional defects when grown in a simplified dermis-like culture system in vitro, we embedded isolated endothelial cells as single cells in a three-dimensional fibrillar collagen matrix and induced the formation of a vascular network with various stimuli. In this culture system, isolated MMP-14 EC-/- endothelial cells formed some tube-like structures similar to controls, but did not penetrate the gels. In these cultures, in the absence of MMP-14, we detected large areas of clustered and flattened cells. Taken together, these studies showed that in vivo deletion of MMP-14 in endothelial cells whereas important for membranous ossification and suture closure in skulls development, is dispensable during skin developmental processes and homeostasis.

P95, Role of fibroblast MMP-14 in skin homeostasis and melanoma growth

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MMP-14 is a membrane-bound matrix metalloprotease that coordinates breakdown of extracellular matrix during tissue remodeling. Mice lacking endogenous production die by 3 weeks of age and present skeletal abnormalities and delayed postnatal growth. To avoid early lethality and to analyze the distinct function of fibroblast MMP-14 in adult skin homeostasis we generated mice with inducible deletion of MMP-14 in the dermal fibroblasts (MMP-$14^{Sf-/-}$). To our surprise when deletion of MMP-14 was induced, mice were smaller than control littermates. Moreover, the animals developed a fibrotic skin phenotype with increasing up to a twofold thickness of the dermal connective tissue. Along with levels of collagen type I, stiffness and tensile strength increased, while collagen cross-links were unaltered. In vitro, MMP-$14^{Sf-/-}$ fibroblast did not display significant enhancement of collagen *de novo* synthesis, but collagen type I accumulated as a result of the loss of collagenolysis by MMP-$14^{Sf-/-}$ fibroblasts. However, bleomycin-induced fibrosis in skin proceeded in a comparable manner in controls and MMP-$14^{Sf-/-}$, but the resolution was impaired in MMP-$14^{Sf-/-}$.
As we know that tumor growth strongly depends on the interaction of tumor cells with their microenvironment including the surrounding connective tissue we were interested to learn whether alteration of the dermal connective tissue could alter proliferation of tumor cells. Surprisingly, grafting melanoma cells to the collagen-rich stiff dermis of these mice resulted in decreased tumor growth. This was paralleled by reduced numbers of lymphatic and blood vessels around the invaded tumors in addition to the reduced proliferation of melanoma cells, while inflammatory responses were not altered. Altogether, loss of MMP-14 activity in fibroblasts results in enhanced tissue density and tension which in turn inhibit melanoma growth by attenuating angiogenesis and tumor cell proliferation.
Taken together, these data indicate that MMP-14 expression in fibroblasts plays a crucial role in collagen remodeling in adult skin and largely contributes to dermal homeostasis underlying its pathogenic role in fibrotic skin disease and during melanoma growth.

P96, Laminin-511 controls melanocyte differentiation by regulating their migration

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Laminins, the major basement membrane (BM) components, are heterotrimeric glycoproteins, composed of α, β and γ chains. In skin, the main laminin isoforms in the dermo-epidermal BM are laminin-332, laminin-511 and laminin-211, the latter being restricted to hair follicles (HFs). Laminin γ1 chain is the most abundant γ chain, deletion of which leads to early embryonic lethality at day E5.5. To elucidate the cellular function of γ1 chain in skin, we generated mice with keratinocyte-specific deletion of this chain (*Lamc1*^{EKO}) by using the keratin 14-Cre/*loxP* system.
Strikingly, *Lamc1*^{EKO} mice show delayed and reduced coat pigmentation. This was not due to a reduction in total melanocyte numbers, but attributable to reduced expression of differentiation specific enzymes TRP-1, TRP-2 and tyrosinase by melanocytes. Since melanocytes in controls differentiate upon migrating into HFs, we postulate that in *Lamc1*^{EKO} skin, the migratory capacity of melanocytes into HFs may be impaired, resulting in an abnormal distribution within the skin. Indeed, melanocytes in skin of *Lamc1*^{EKO} mice were retained in the epidermis and were less abundant in HFs. The observed defective melanocyte migration was explained by the loss of keratinocyte-derived laminin-511 and ectopic deposition of fibroblast-derived laminin-211 in the whole dermo-epidermal BM of *Lamc1*^{EKO} mice. This is concordant with our finding that laminin-511, but not laminin-211, is the preferred substrate for migration and adhesion of primary melanocytes *in vitro*.
Besides an appropriate extracellular matrix supporting migration, melanocyte migration into HFs also depends on the chemoattractant SDF1 (Stromal cell-derived factor 1), which acts through its receptor CXCR4 being expressed on melanocytes. Interestingly, laminin-511, but not laminin-211, induces the expression of CXCR4 on melanocytes, required for SDF1-mediated migration. By contrast, total expression levels of SDF1 in skin of *Lamc1*^{EKO} mice did not differ from controls.
In summary, we show that laminin-511 regulates the differentiation of melanocytes by two independent mechanisms that control their migration from the epidermis into HFs. First, laminin-511 serves as migration substrate. Second, it stimulates the expression of CXCR4 on melanocytes and their recruitment into HFs in an SDF1-dependent manner.

P97, The gC1q domain is responsible for EMILIN1-induced lymphangiogenesis

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Introduction
Lymphatic vessels (LVs) play a pivotal role in the control of tissue homeostasis and have also emerged as important regulators of immunity, inflammation and tumor metastasis. EMILIN1 is the first ECM protein identified as a structural modulator of the growth and maintenance of LV; accordingly, *Emilin1*$^{-/-}$ mice display lymphatic morphological alterations leading to functional defects (mild lymphedema, leakage and compromised lymph drainage). Many EMILIN1 functions are regulated by the ligand-receptor interaction of its gC1q domain: the E933 within a protruding loop of the gC1q structure is the site of interaction with α4/α9β1 integrins and, in contrast to a large body of evidence that signals generated by ligand-activated integrins are pro-proliferative, gC1q binding reduces cell proliferation.

Materials and Methods.
We produced E933A EMILIN1 transgenic mice (E933A-TG), expressing a mutant EMILIN1 unable to be engaged by integrin receptors. Morphological analyses and lymphangiography assays were then performed to investigate the specific regulatory control of the gC1q domain on lymphangiogenesis, together with in vitro tubuligenesis tests.

Results
E933A-TG presented abnormal architecture of LVs with dense, tortuous and irregular networks; moreover, the number of anchoring filaments was reduced and collector valves displayed aberrant narrowed structures. E933A mutation affects also lymphatic function in lymphangiography assays and makes TG mice more prone to develop LN metastasis. The hypothesis that gC1q is the most important domain to induce a correct lymphangiogenesis response was confirmed and reinforced by functional *in vitro* tubuligenesis tests (in which EMILIN1, gC1q WT but not E933A mutant recombinant stimuli were able to organize LECs in a 3D-network of tubes). In addition, *ex vivo* thoracic-duct ring assay revealed that TG-derived LECs show severely reduced sprouting capacity and are not able to organize into capillary-like structures.

Discussion
All both morphological and functional evidences, describing for E933A-TG mice a lymphatic phenotype very similar to that already observed for the *Emilin1*$^{-/-}$ background, clearly indicate that the most important EMILIN1 element able to induce a correct lymphangiogenesis response is within the C-terminal domain gC1q and that it involves the interaction with the integrin.

P98, Lymphoid like extracellular matrix partially polarize stroma cells toward a lymphoid phenotype

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Introduction
Lymph nodes are organized into different niches to support efficient encounter between lymphocytes and antigens. Those niches are structured by lymphoid stromal cells (LSC) that synthesise specific extracellular matrices (ECM). It has been shown that LSC modulate B cells migration, survival and antigen encounter. However, little is known on the role of ECM in lymph nodes. This project aim to assess the effect of ECM on the polarization of stromal cells.

Materials and Methods
Tonsil Stromal Cells (TSC) and Adipose Derived Stromal Cells (ADSC) were cultured during ten days with or without TNFa and LTa1b2 to induce a LSC phenotype. Cells were then decellularized to remove cells but not the ECM. TSC and ADSC were then cultured for three days on ECMs and lymphoid stromal cells markers (ICAM-1, VCAM-1 and GP38) expressions were evaluated by flow cytometry.

Results
Flow cytometry assays show an increase expression of ICAM-1 on both stromal cells types (TSC and ADSC) only when they are cultivated on lymphoid like ECMs produced by ADSC polarized into LSCs. In contrast, VCAM-1 and GP38 expressions do not change.

Discussion
These first results show that lymphoid like ECM can modify stromal cells expression for lymphoid stromal cells marker. This suggests a mechanism of LSC self-induction in lymphoid organ, with ECM microenvironment encouraging stromal cell polarization to LSC phenotype. Despite those encouraging results, the ECM effect on stromal cells polarization remain unclear and further analyses are required to better understand this phenomenon. Tests on B cells are currently been made to assed ECM influence on their survival and proliferation. Moreover, RNAseq anlaysis on different stromal subsets in human are ongoing. It will help us to identify specific ECM molecules that could play a role in the adaptive immune response.

P99, BMPER cleavage in signalling and disease

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Introduction
Bone morphogenic protein-binding endothelial regulator protein precursor (BMPER) is an extracellular matrix glycoprotein that regulates BMP activity. BMPER-mediated BMP activation and inhibition are concentration and context-dependent. BMPER is cleaved at Gly-Asp-Pro-His (GDPH) site where the cleavage products remain attached through a disulphide bond. Mutation of the cleavage site from a Proline to Leucine at Position 370 (P370L) in humans leads to skeletal disorders Diaphanospondylodysostosis (DSD) and Ischiospinal Dysostosis (ISD). However, the cleavage mechanism of BMPER and the functional role of BMPER cleavage is still unclear. Since BMP regulation is tightly regulated by two other extracellular glycoproteins Chordin and Twisted gastrulation (Tsg), we aim to investigate how other matrix proteins affect the BMPER or P370L-regulated BMP signalling pathway.

Materials and Methods
BMPER and BMPER-P370L expression in stable lentiviral-transduced Human Umbilical Vein Endothelial Cells (HUVECs) was confirmed using western blot. Nickel-affinity and size exclusion chromatography was used to purify recombinant WT and P370L-BMPER from conditioned media of stably transduced Human Embryonic Kidney 293 (HEK293) EBNA cells. Stable chondrogenic ATDC5 cells transduced with BMP reporter were used to study BMPER-mediated BMP regulation. Recombinant BMPER and P370L, were added in addition to BMP2, to distinguish their role in BMP activity.

Results
BMPER and P370L were expressed in HEK293 EBNAs and HUVECs. P370L-BMPER did not undergo intracellular cleavage but was susceptible to an extracellular protease. BMPER is a BMP2 antagonist and cleavage was not required for its inhibitory function.

Discussion
This study confirms that BMPER inhibits BMP2 signalling. Both BMPER and P370L are strong inhibitors of BMP2, hence it would also be interesting to associate the role of BMPER and P370L in the regulation of other BMP isoforms. As P370L mutation at the cleavage site did not affect the inhibition profile of BMPER, our next goal is to investigate how the addition of Tsg alters the effect of BMPER or P370L in BMP inhibition. This study will improve our understanding of the functional significance of BMP signalling and how the pathway is regulated extracellularly.

P100, Regulation of Metalloproteinases during Ovulation in Zebrafish

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Ovulation requires proteinases to promote the rupture of ovarian follicles. However, the identity of these proteinases remains unclear. In our previous studies using RNA-seq analysis of differential expressed genes, we found significant down-regulation of five metalloproteinases: *adam8b* (a disintegrin and metalloproteinase domain 8b), *adamts8a* (a disintegrin and metalloproteinase with thrombospondin motif 8a), *adamts9*, *mmp2* (matrix metallopeptidase 2), and *mmp9* in the nuclear progestin receptor knockout (*pgr*$^{-/-}$) zebrafish that have failed to ovulate. We hypothesize that these metalloproteinases are responsible for ovulation and are regulated by progestin. In this study, we first determined the expression of these five metalloproteinases and *adamts1* in preovulatory follicles at different times within the spawning cycle in *pgr*$^{-/-}$ and wildtype (*wt*) zebrafish and under varying hormonal treatments. We found that transcripts of *adam8b*, *adamts1*, *adamts9* and *mmp9* increased drastically in the preovulatory follicular cells of *wt* female zebrafish, while changes of *adamts8a* and *mmp2* were not significant. This increase of *adam8b*, *adamts9* and *mmp9* was significantly reduced in *pgr*$^{-/-}$, whereas expression of *adamts1* was not affected in *pgr*$^{-/-}$ zebrafish. Interestingly, expression of two key upstream regulators, *pgr* and the luteinizing hormone/choriogonadotropin receptor (*lhcgr*), increased sequentially in preovulatory follicular cells in *wt* fish prior to the increase of metalloproteinases *in vivo*. Thereafter, we focused on hormonal regulation of the *adamts9* proteinase because of its specific expression in the follicular cells. Strong immunostaining of Adamts9 was observed in the follicular cells of *wt* fish, and this expression was reduced drastically in *pgr*$^{-/-}$. Importantly, preovulatory follicles markedly increased *adamts9* expression in a dose, time and Pgr-dependent manner when stimulated by 17□,20□-dihydroxy-4-pregnen-3-one (DHP, a progestin) *in vitro*. Exposure to human chorionic gonadotropin (hCG) alone had no effect on expression of *adamts9* or oocyte maturation. Upon steroid (DHP, RU486, or testosterone) induced oocyte maturation, hCG could further stimulate *adamts9* expression both in *wt* and *pgr*$^{-/-}$zebrafish. Our results provide the first evidence that hormonal upregulation of *adamts9* occurs specifically in preovulatory follicular cells of zebrafish prior to ovulation. *Adamts9* is primarily regulated by progestin and then reinforced by gonadotropin via their cognate receptors.

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Key words: ovulation, adamts9, gonadotropin, progestin, Pgr, hCG

Workshop 5: ASMB sponsored session: Pathobiology and Therapeutics to Fibrosis

P101, Scleraxis plays an indispensable contribution to progenitor lineage direction in adult tendon wound healing

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Introduction
Tendon is a dense connective tissue that transmits high mechanical forces from skeletal muscle to bone. Adult tendon injuries occur very frequently, but injured tendon heals very slowly and the mechanisms of the slow-healing response to injury are still largely unknown. A transcription factor Scleraxis (Scx) is a highly specific marker of precursor and mature tenocytes. Mice lacking *scx* show a specific and virtually complete loss of tendons during development. However, the functional contribution of Scx to adult tendon wound healing has not yet been fully characterized.

Materials and Methods
We developed a simple and reproducible Achilles tendon 'partial-transection' injury model, and utilized a combination of *ScxGFP*-tracking and loss-of-function systems. Mouse adult tendon progenitor cell lines were generated from adult *scx(flox/flox)/ScxGFP* mouse Achilles tendon under a *Trp53*- and *Cdkn1a* (*p21)*-null genetic background.

Results
We show here that paratenon cells, representing a stem cell antigen-1 (Sca-1)-positive, Scx-negative progenitor subpopulation, display Scx induction, migrate to the wound site and produce extracellular matrix (ECM) to bridge the defect, whereas resident tenocytes exhibit a delayed response. The induction of Scx in the progenitors is initiated by TGF-β-signaling. The *scx*-deficient mice had migration of Sca-1-positive progenitor cell to the lesion site following injury, but impaired ECM assembly to bridge the defect. Mechanistically, scx-null progenitors displayed higher chondrogenic potential with up-regulation of SRY-box 9 (Sox9) coactivator PPAR-gamma coactivator 1α (PGC-1α) *in vitro*, and knock-in analysis revealed that forced-expression of full-length *scx* significantly inhibited *sox9* expression. Accordingly, *scx*-null wounds formed cartilage-like tissues that developed ectopic ossification.

Discussion
Our comprehensive studies of adult tendon wound provide the following compelling evidence: 1) Scx plays indispensable roles in proper healing following adult tendon injury; 2) There is a direct link between tendon progenitor cell-lineage mediated by Scx and adult tendon pathology; and 3) Certain Sca-1-positive progenitor subpopulations identified in the paratenon could provide novel targets to develop strategies to facilitate tendon repair. We propose that the regulatory mechanisms underlying lineage-specific differentiation in adult tissue progenitors shown here could be translated in a broader variety of tissues or systems in the body.

P102, ST: The importance of tissue transglutaminase for the deposition of matrix proteins in idiopathic pulmonary fibrosis

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Introduction
Idiopathic pulmonary fibrosis (IPF) is a progressive and life-threatening disease that has 5000 new cases diagnosed each year in the UK with a median survival of less than 3 years. Previous studies show tissue transglutaminase (TG2) expression and activity is elevated in the lungs of IPF patients, however the role of TG2 in IPF has not yet been elucidated. TG2 is a multifunctional enzyme that has been implicated in various pathological conditions. This study aims to validate TG2 as a therapeutic target for IPF.

Materials and Methods
Primary Normal Human Lung Fibroblast (NHLF) and fibroblasts derived from patients with IPF were cultured in DMEM supplemented with 10% FCS and 1% NEAA. NHLF cells were transduced using a WT-TG2 or catalytically inactive TG2 (C277S) carrying lentivirus. Protein levels in whole cell lysates and the ECM were measured via Western blotting. Extracellular fibronectin and collagen was measured using immunocytochemistry and high-content imaging. TGFβ activity levels were measured using a Mink Lung Epithelial Cell (MLEC) TGFβ reporter assay. CRISPR-Cas9 genome editing was performed on IPF fibroblasts targeting the *TGM2* gene.

Results
These data show that stimulation of NHLF with TGFβ1 markedly increases TG2, collagen and fibronectin deposition into the matrix. The addition of exogenous TG2 elevates TG2, fibronectin and TGFβ1 deposition into the matrix, whilst also increasing levels of active TGFβ. The lentiviral transduction of WT-TG2 into NHLF cells increases matrix deposition of fibronectin and TGFβ1, however this is ameliorated in NHLF cells transduced with a C277S-mutant TG2. The application of a cell-impermeable TG2-specific inhibitor prevents TGFβ1-induced increases in TG2 and fibronectin deposition in NHLF cells, whilst also decreasing TG2 and fibronectin matrix deposition in IPF fibroblasts. Finally, CRISPR-Cas9 genome editing targeting *TGM2* in IPF fibroblasts leads to a reduction in collagen deposition.

Discussion
This study demonstrates that TG2 plays a vital role in the deposition of collagen, fibronectin and TGFβ1. Further to this, these data demonstrate that it is extracellular, catalytically-active TG2 that is required to elevate matrix deposition of proteins in healthy lung fibroblasts. Most importantly, targeting TG2 in IPF fibroblasts leads to a marked reduction in matrix protein deposition.

P103, ST: Ubiquitous removal of ccn2 in a bleomycin induced pulmonary fibrosis model is detrimental to survival

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Introduction
Idiopathic pulmonary fibrosis (IPF) is a chronic progressive form of lung fibrosis that is ultimately fatal in all cases. Mortality rates at 5 years post diagnosis of IPF are only 20%. Current treatment options fail to prevent progression or cure this disease. Fibrotic lung disease is characterised by over-production and deposition of collagen I, however the mechanism behind the initiation and propagation of this process is unknown.
CCN2 has been shown to be upregulated in patients with IPF and in animal models of pulmonary fibrosis. We tested whether the ablation of CCN2 from fibroblasts or all cells would ameliorate the pathology.

Materials and Methods
We mated a floxed CCN2 mouse line with either of two inducible CreER^{T2} recombinase transgenic mouse lines to examine the effects of removing CCN2 in a fibroblast specific (Col1a2-cre) and ubiquitous (ROSA26-cre) manner, followed by a bleomycin-induced model of IPF. Bleomycin model was carried out via oropharyngeal aspiration route with a single dose of 0.375 ng/g bleomycin administered in sterile PBS. Lungs were compared with untreated mice using a Bruker Skyscan 1272 ex-vivo µCT scanner to identify regions of tissue deposition and compare air with tissue densities. A single lobe from each lung was analysed histologically to confirm the fibrotic pathology and tissue composition. RNA was extracted from the remaining lobes for qPCR analysis. Changes in gene expression were determined using a $\Delta\Delta$CT calculation.

Results and Discussion
The data showed that the removal of CCN2 from fibroblast cells did not provide protective effect at day 14 post bleomycin induced pulmonary fibrosis. However, when CCN2 was removed ubiquitously it resulted in a severe fibrosis with an accelerated rate of matrix synthesis and deposition. This suggested that in vivo, CCN2 plays a regulatory role to limit the rate of matrix production, rather than being the culprit molecule as suggested in the literature. Therefore, we conclude that any attempts to block CCN2 universally would be detrimental to lung architecture and function.

P104, Differences between the matrix of the neonatal and adult extrahepatic bile duct: implications for injury

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Introduction
The submucosal interstitial space of the extrahepatic bile duct (EHBD) is a poorly characterized space that is known to contain organized bands of collagen and fluid flow in adults. Its structure in neonates is not known, but may be important in diseases such as biliary atresia that specifically affect the neonatal EHBD. We carried out a detailed analysis of the ECM of the neonatal compared to the adult mouse EHBD in an effort to identify anatomical features that might render neonates particularly susceptible to injury.

Materials and Methods
EHBDs were isolated from adult mice and pups at postnatal days 0-15. These were imaged using transmission electron microscopy, second harmonic generation microscopy and were stained for matrix components and cell type markers.

Results
The submucosal space of EHBDs in adult mice consists of collagen I/III bundles, proteoglycans, hyaluronic acid and elastin, interspaced with fluid-filled spaces. The collagen bundles are lined by fibroblasts. Conversely, the neonatal EHBD interstitium has minimal elastin and collagen; these are progressively deposited in the first 15 days of life. The diameter of collagen fibrils and expression of the fibrillogenesis-associated proteoglycans lumican and fibromodulin change in parallel during postnatal development. Neonatal submucosal cells are more rounded and contain large amounts of rough endoplasmic reticulum, suggesting that they are responsible for collagen deposition. Many of these cells in the neonate stain for myofibroblast marker αSMA and mesenchymal stem cell marker CD105.

Discussion
The extracellular matrix and phenotype of cells present in the submucosal space of the EHBD significantly changes from birth to adulthood. The early presence of a space filled primarily with proteoglycans and lacking major structural proteins of the matrix suggests that neonatal EHBDs will have poor response to mechanical stress (e.g. obstruction) and that injurious material (e.g. bile, toxins, viruses) is more likely to spread within the submucosal interstitium. The presence of a larger αSMA-positive and metabolically-active cell population raises the possibility that the neonatal EHBD is primed to respond to injury and fibrotic cues. Future work will focus on characterising the mechanical responsiveness and fluid flow of the neonatal vs. adult duct and on assessing the fibrogenic cell population at different stages of development.

P105, A 3D model of human mature lysyl oxidase

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Introduction
Lysyl oxidase (LOX, protein-lysine 6-oxidase) catalyses the oxidative deamination of lysyl and hydroxylysyl residues in collagens and elastin, and the first step of their covalent cross-linking. LOX expression is up-regulated in fibrosis and metastasis. No 3D structure of the catalytic domain of LOX is available, preventing the design of specific inhibitors. This prompted us to build a 3D model of human LOX.

Materials and Methods
Robetta was used to build fragment libraries of 3 and 9 residues based on the structures available in the Protein Data Bank, and distant homologs of the human LOX sequence based on hidden Markov models were retrieved with HHpred. 5,000 models were generated by Rosetta using Robetta fragment libraries, selected templates from HHpred, and structural features (copper ion, the lysyltyrosylquinone - LTQ - cofactor and disulfide bonds). Long molecular dynamics simulations were run to assess the model stability with Gromacs software and the amber99SB-ildn*-q force field including modified parameters for copper ions.

Results
The model has a V-shaped structure with a groove, which contains the catalytic site in close contact with LTQ, and can accommodate a collagen triple-helix. The groove acts as a hinge axis
leading either to an open conformation, where the catalytic site is accessible, or to a closed one where it is buried. This motion is supported by the fact that the radius of gyration of the model is correlated with the hinge angle.

Discussion This model is the first one to recapitulate all known molecular features of LOX (the copper ion coordinated by three histidine residues, LTQ and five disulfide bridges). It will be useful to perform docking experiments with LOX substrates and other partners to decipher its molecular mechanisms of action, and to design new LOX inhibitors for therapeutic purpose.

P106, Novel non-invasive biomarkers of extracellular matrix remodeling reflect the burden of renal fibrosis in histological specimens and are associated to renal function.

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Introduction
Renal fibrosis is a common hallmark of several indications of chronic kidney disease (CKD). Patients with more advanced and active fibrogenesis are more likely to progress to end-stage renal disease, a condition that presents a high risk of mortality and the dependance on dialysis or renal transplantation for survival.
The gold standard for evaluation of renal fibrosis is at the moment renal biopsy, a procedure presenting numerous drawbacks. It is therefore crucial to develop novel methods to detect and assess fibrosis in the renal tissue, in order to timely diagnose and stop the disease and to help the development of anti-fibrotic treatment.

Materials and Methods
Here we quantified the concentration of novel biomarkers of fibrosis, namely a fragment of collagen type III generated by MMP-9 (C3M), reflecting interstitial matrix degradation, and a fragment of the α3 chain of collagen type VI C5 domain, representing collagen type VI formation, in serum and urine of patients with IgA nephropathy (n=49) and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) (n=47). Renal biopsies taken at the time of sample collections were evaluated for extent of fibrosis by an experienced pathologist.

Results
Both serological and urinary levels of PRO-C6 correlated with percentage of histological fibrosis (Spearman $r=0.51$, $p<0.0001$ and Spearman $r=0.35$, $p=0.0009$, respectively) and could separate patients with the most severe fibrosis stages as evaluated by the Banff score (ci0 and ci1 vs ci 3, $p<0.01$, and $p<0.05$ respectively). Urinary levels of C3M inversely correlated with percentage of histological fibrosis (Spearman $r=-0.43$, $p<0.0001$) and levels were the lowest in patients with the most advanced fibrosis stages (ci0 and ci1 vs ci 3, $p<0.05$).
Moreover, PRO-C6 in serum and urine gradually increased in increasing CKD stages, while urinary C3M gradually decreased in increasing CKD stages (Kruskall-Wallis $p<0.0001$ for all markers).

Discussion
PRO-C6 and C3M were previously established as promising prognostic marker for adverse outcome in CKD in several independent cohorts. Here we showed that these markers can actually reflect the burden of fibrosis and possibly the activity of the fibrogenic process happening in the kidneys.

P107, ST: Inhibition of heparanase protects against renal failure and fibrosis following ischemia/reperfusion.

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Introduction
Renal ischemia/reperfusion (I/R) injury occurs in patients undergoing renal transplantation and with acute kidney injury (AKI). It is responsible for the development of chronic allograft dysfunction characterized by parenchymal alteration and fibrosis. Heparanase (HPSE), an endoglycosidase that regulate EMT and macrophages polarization, is an active player in the biological response triggered by ischemia/reperfusion (I/R) injury.

Materials and Methods
I/R was induced in vivo by clamping left renal artery for 30 min in wt C57BL/6J mice. Animals were daily treated or not with Roneparstat (an inhibitor of HPSE) and sacrificed after 8 weeks. HPSE, fibrosis, EMT-markers, inflammation and oxidative stress were evaluated by biomolecular and histological methodologies together with the evaluation of renal histology and measurement of plasmatic and urine parameters of renal function.

Results
8 weeks after I/R HPSE was upregulated both in renal parenchyma and plasma whereas tissue specimens showed clear evidences of renal injury and fibrosis (IF/TA). The inhibition of HPSE with Roneparstat restored histology and fibrosis level comparable with that of control. I/R injured mice showed a significant increase of the EMT, inflammation and oxidative stress markers but they were significantly reduced by the treatment with Roneparstat. Finally, the inhibition of HPSE in vivo almost restored renal function as measured by BUN, plasma creatinine and albuminuria.

Discussion
The present study point out that HPSE is actively involved in the mechanisms that supervise the development of renal fibrosis arising as a consequence of the ischemia-reperfusion damage. In the transplanted organ HPSE inhibition would therefore constitute a new pharmacological strategy to reduce acute kidney injury and to prevent the chronic pro-fibrotic damages induced by I/R.

P108, PDE5 inhibitors and selective oestrogen receptor modulators exert anti-fibrotic synergy in *in vitro* and *in vivo* models of Peyronie's disease

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Introduction
Peyronie's disease (PD) is characterized by fibrosis in the penile tunica albuginea (TA) leading to pain, curvature and erectile dysfunction. Currently treatment is almost limited to surgery, highlighting the need for novel medication.

Materials and Methods
A novel high-throughput phenotypic screening assay measuring TGF-β1 induced myofibroblast transformation derived from human TA was developed. This assay was then used to screen FDA-approved drugs using In-Cell ELISA (ICE) method which revealed hits from two classes, selective oestrogen receptor modulators (SERMs) and PDE5 inhibitors (PDE5i). These were further confirmed using functional assays measuring contraction and ECM formation. The hits and their combination were investigated in vivo using an animal model of PD. Sprague-Dawley rats were divided in 5 groups: 1) TGF-β1 injection in the TA (TGI) 2) vehicle injection, 3) TGI plus daily PDE5i (vardenafil), 4) TGI plus daily SERM (tamoxifen), 5) TGI plus daily combination of PDE5i and SERM. Five weeks after injection +/- treatments the rats were subjected to erectile function measurement with subsequent molecular analysis of the penis.

Results
The fibroblast identity of human TA derived cells was confirmed. ICE was able to reproducibly quantify TGF-β1-induced transformation of fibroblasts to myofibroblasts. Five FDA approved drugs (three PDE5i and two SERMs) significantly inhibited TGF-β1-induced myofibroblast transformation in a concentration dependent manner. The drugs were capable of decreasing collagen contraction, as well as ECM formation and synergised in inhibiting TGF-β1-induced myofibroblast transformation and collagen contraction. *In vivo* data revealed that TGF-β1 injection caused fibrosis in the penis of rats which resulted in erectile dysfunction, ECM accumulation and smooth muscle loss. These effects were prevented in the groups which received a PDE5i or SERM. The drug combination showed a synergistic effect on various hallmarks of fibrosis.

Discussion
An anti-fibrotic effect could be confirmed for PDE5i, SERM, and their combination *in vitro* and *in vivo* on a functional, histological and molecular level. The drugs synergise both *in vitro* and *in vivo*, suggesting a potential combination therapeutic approach for early phases of PD and possibly for other fibrotic diseases.

P109, Proteoglycan-integrin interaction drive pathological cardiac remodelling

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Introduction
Heart failure is a condition with very poor prognosis affecting 26 million people globally, with no current effective therapy. A key driver of heart failure is pressure overload, which results in cardiac fibrosis and a deleterious stiffening of the left ventricle. Proteoglycans and integrins represent novel members of the mechanosensing apparatus of the heart, crucial to detecting alterations in the mechanical stress the heart is exposed to. Determining the key mechanoreceptors involved in pathological cardiac remodelling is critical to identifying new therapeutic targets.

Materials and Methods
We generated genetically modified mice lacking expression of syndecan-4 (SDC4), integrin α11β1 (Itgα11) and a double knockout strain. Mice were subjected to aortic banding to induce cardiac pressure overload and phenotyped over a period of 2 weeks. In vitro experiments were conducted on a mouse fibroblast cell line (NIH 3T3) to elucidate the functional effects of SDC4 and Itgα11 expression on fibrosis.

Results and Discussion
$SDC4^{-/-}$ and $Itgα11^{-/-}$ mice still developed cardiac hypertrophy and fibrosis following surgical induced pressure overload comparable to wild type (WT) mice at the 2 week time-point, however $SDC4^{-/-}Itgα11^{-/-}$ double knock-out mice showed reduced pathological remodelling, including reduced fibrosis. We attribute this protective phenotype to a functional overlap between SDC4 and Itgα11 receptors. Through in vitro experiments, we determined a reciprocal pattern of expression between these receptors under different stimulations, gene silencing and overexpression assays, suggesting single targeting of these receptors is ineffective. Further, we suggest a novel mechanism by which SDC4 regulates the surface expression of Itgα11 mediated through endosomal trafficking. Together our results indicate that the dual targeting of these receptors may be of therapeutic benefit to patients at risk of developing pressure overload induced heart failure.

P110, ST: Extra-cellular matrix induced by aldosterone through a G-protein coupled receptor revealed in a novel Drosophila model of renal fibrosis

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Introduction
Extra cellular matrix accumulates in the course of renal fibrosis. One potential entry to modulate this pathology is through control of steroid hormones. Notably human aldosterone appears to foster fibrosis when it is chronically expressed, as in diabetes. Aldosterone may affect fibrosis through the mineralocorticoid nuclear hormone receptor, yet some reports implicate an unidentified, non-canonical receptor. With a novel Drosophila model system of fibrotic disease and ECM accumulation, we describe such an alternative in the form of a G-protein coupled receptor, Drosophila dopEcR.

Materials and Methods
To quantify renal fibrotic disease in Drosophila we measured protein secretion in urine (proteinuria) and impaired nephrocyte filtration (glomerular disease). Extra-cellular matrix pericardin (collagen IV) was quantified as mRNA from excised nephrocyte/cardia tissue, by immunostaining, and by Western analysis. We fed human aldosterone and Drosophila steroid hormones (ecdysone and 20-hydroxyecdysone) to adults for 3 w to test their chronic impact on fibrosis. With tissue specific drivers, we expressed RNAi against candidate genes to identify the receptor and tissue responsible for steroid-induced fibrosis.

Results
Aldosterone and ecdysone strongly induce fibrosis in the Drosophila renal system, measured as proteinuria, impaired nephrocyte filtration, and accumulation of ECM collagen (pericardin). All pathology arose from pericardin produced by cardia-myocytes. Steroid induce fibrosis was not ameliorated by RNAi against the Drosophila canonical nuclear hormone receptor EcR, but all were blocked by knockdown of dopEcR, a membrane associated G-protein coupled receptor.

Discussion
Human aldosterone and Drosophila ecdysone signal through a novel GPCR to modulate ECM accumulation and fibrotic pathology of the renal system. The GPCR dopEcR is a dual ligand receptor responsive to dopamine and ecdysone, previously described to affect select neuronal behaviours. We find it also acts in heart muscle cells, perhaps reflecting myofibroblast-like function, to structure expression of pericardin (collagen IV) in the cardia-renal tissue. Chronic exposure to aldosterone or ecdysone stimulates excess ECM and disrupts both renal and heart function. Potential homologs of dopEcR occur in humans, and may present targets for pharmacological approaches to repress fibrotic disease.

P111, Deciphering the matrix protein 'fingerprint' in Dupuytren's fibrosis

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Introduction
Dupuytren's contracture is a common fibrosis of the palmar fascia of the hand associated with collagen accumulation, an altered expression of matrix metalloproteinases (MMPs) and myofibroblast-mediated matrix compaction. There are increased collagen and extracellular matrix mRNAs in Dupuytren's and a loss of specific regulatory miRs that dampen production of these mRNAs. There can however be less than a 50% correlation between mRNA and protein abundance and a previous proteomics study identified pro-survival cellular signaling pathways in Dupuytren's, but few alterations in matrix proteins.

Materials and Methods
Dupuytren's tissue (n=5) was obtained following surgery to treat digit contracture and separated into active 'nodule' and scar-like 'cord' regions. Control tissue samples (n=5) were derived from the carpal ligament of carpal tunnel decompression patients. Tissues was obtained with REC/HRA ethical approval and patient consent. Tissues were labelled overnight in lysine-deficient medium supplemented with heavy (13C) lysine. Labelling media was reserved and tissue extracted using a sequential guanidine/RapiGest method, with and without prior chondroitinase ABC treatment. Samples were analysed by mass spectrometry and data processed using Mascot, Xcalibur and Peaks software.

Results
Label-free analysis identified differences in collagen and proteoglycan composition between tissues. 13C-labelled peptides were primarily identified in the explant media. 13C-labelled type I collagen, matrix metalloproteinase 2 (MMP2) and fibronectin were present in the media of Dupuytren's explants, whilst both tissue types synthesised matrix metalloproteinase-3 (MMP3), tissue inhibitor of matrix metalloproteinases 2 (TIMP2) and insulin-like growth factor binding protein 7 (IGFBP7).

Discussion
Continual production of the structural extracellular matrix proteins type I collagen and fibronectin occurs in Dupuytren's tissue explants, which may be the underlying cause of matrix accumulation and disease recurrence following surgical treatment. Furthermore TIMP2 may become saturated by excess MMP2 production in Dupuytren's disease and be unable to counteract pathological extracellular matrix remodelling.

Workshop 6: Mechanisms of Matrix Disease

P112, Vascular calcification during chronic kidney disease: role of the RAGE/Cathepsin S/elastin peptides axis

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Introduction

Vascular calcification is a common feature of patients with chronic kidney disease (CKD). We recently reported a role for the Receptor for Advanced Glycation End products (RAGE) in the uremic vascular calcification process following engagement by uremic toxins. Moroever, several studies have suggested the involvement of Cathepsin S in elastolysis and vascular calcification during CKD. However, the link between these actors and the mechanisms implicated and the putative role of RAGE in Cathepsin S expression and the subsequent elastolysis are unknown.

Materials and Methods

We used a mouse model of uremic vasculopathy in the ApoE-/- or ApoE-/-/RAGE-/- (DKO) backgrounds as well as primary cultures of VSMCs isolated from C57Bl6J mice.

Results

We found that induction of CKD increases the calcifications processes in the cardiac valves of ApoE-/- mice whereas DKO are protected. Moreover, aortas analysis showed that Cathepsin S expression and elastolysis seem to be greater in ApoE-/- than in DKO animals. Using recombinant Cathepsin S, we showed by using electron microscopy scanning and mass spectrometry analysis that this protease degrade insoluble elastin producing bioactive Elastin Derived Peptides (EDPs). Then, we showed that *in vitro* calcification process is triggered when VSMCs are incubated with inorganic phosphate and is increased in the presence of EDPs. At last, the use of an inhibitor of the Elastin Receptor Complex (ERC), DANA, abolished this phenomenon.

Discussion

In conclusion, during CKD, activation RAGE by uremic toxins leads to Cathepsin S expression promoting elastolysis leading to the production of bioactive EDPs. These peptides accelerate vascular calcification by binding on ERC. This study highlights the potential for targeting the ERC to modulate vascular calcification during CKD.

P113, Andrographolide inhibits osteoclastogenesis by suppressing ERRα and prevents ovaractomy and obesity induced bone loss

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Introduction
Osteoclasts play pivotal roles in the regulation of bone homeostasis. Atypical increases in osteoclast activity cause an imbalance in bone remodeling that leads to bone diseases. Estrogen-related receptor α (ERRα) is an orphan nuclear hormone receptor that regulates various metabolic processes. Osteoclasts undergo metabolic adaptations to meet the increased bio-energetic demands of the energy consuming process of bone resorption. Glutaminase converts glutamine to glutamate that is further catabolized through TCA cycle for ATP production. We hypothesized that ERRα coordinates with PGC-1β to mediate such metabolic transition that advances osteoclast differentiation. Andrographolide, a diterpene lactone compound extracted from the leaves of Acanthaceae paniculata, might suppress osteoclast differentiation by modulating ERRα activity.

Material and Methods
Bone marrow monocytes were isolated and differentiated into osteoclasts. Cells were infected with ERRα or Glutaminase-expressing lentivirus, and simultaneously treated with or without andrographolide. Luciferase reporter assay, real-time PCR and Western blot were used to analyze the transcriptional regulation and expression levels. Qvariectomized female mice and high fat diet-treated male mice were orally gavaged with andrographolide daily for 4 weeks. Bone analyses were performed by micro-computed tomography and histochemistry.

Results
ERRα coordinated with PGC1β to mediate Glutaminase expression and then facilitated osteoclast differentiation. Andrographolide suppressed osteoclast differentiation by acting as an ERRα modulator. Furthermore, andrographolide suppressed *in vivo* osteoclast formation and prevented estrogen deficiency and high fat diet induced bone loss.

Discussion
These data provide evidence for an underlying mechanism by which ERRα/PGC1β enhances osteoclastogenesis and affects bone homeostasis. This study also highlights that pharmacological inhibition of ERRα maybe further developed into novel treatments for human bone disorders such as osteoporosis.

P114, The Xbp1 branch of the UPR is crucial for alleviation of protein aggregation in cartilage health and disease

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Introduction
Multiple epiphyseal dysplasia type 5 (MED; EDM5) is an autosomal dominant skeletal dysplasia characterised by short-limbed dwarfism. EDM5 results from mutations in *MATN3*, leading to misfolding and intracellular aggregation of mutant matrilin-3 protein, thus triggering an unfolded protein response (UPR) in growth plate chondrocytes. The expression of mutant matrilin-3 elicits a specific disease signature comprising up-regulation of numerous chaperones, PDIs and novel foldases, and the alternative splicing of Xbp1 (Xbp1s).

Material and Methods
In order to study the role of Xbp1s in the pathobiology of EDM5, MED (*Matn3* V194D) knock-in mice were crossed with the cartilage specific *Xbp1* null line and the resulting phenotype was analysed using bone measurements, histology, immunohistochemistry and quantitative methods to assess proliferation and apoptosis. Microarray analysis was used to generate a full transcriptomic profile of the double mutant and the parent lines, all of which were generated on the C57BL/6J background.

Results
Both parent lines presented with a mild to moderate decrease in bone lengths and chondrocyte proliferation. Interestingly, double mutant mice presented with abnormal bell-shaped thoraxes and dramatically reduced bone lengths, severe growth plate disorganisation and dramatically decreased chondrocyte proliferation (66%), suggesting a synergistic effect. In contrast, the metaphyseal dysplasia type Schmid (MCDS) mouse model with a N617K mutation in *Col10a1* presents with a similar disease signature, yet the cartilage deletion of Xbp1 had no effect on the MCDS phenotype. The retention of mutant matrilin-3 appeared increased upon Xbp1 deletion, whilst the levels of several chaperone proteins were noticeably decreased. Microarray analysis revealed that the MED signature genes regulated by Xbp1 were specifically involved in modulating cell stress and cell integrity. Furthermore, data mining was used to compare the levels of UPR in chondrocytes in different zones of the cartilage growth plate and revealed a differentiation state specific response of the cells to both physiological and pathological stress.

Discussion
To summarise, Xbp1s arm of the UPR plays a protective role in the pathobiology of EDM5 but not MCDS, suggesting a proliferation zone specific chondroprotection. The Xbp1s signalling pathway may therefore represent a novel target for the therapy of a subset of aggregation conditions.

P115, ADAMTS7 substrate and cleavage site specificity

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Introduction
The metalloprotease ADAMTS7 has been implicated in the aetiology of coronary artery disease. However, little is known about its physiological function or its substrate specificity. We aimed to characterise the biochemical properties of ADAMTS7 through identification of proteolytic substrates and their respective cleavage site(s).

Materials and Methods
ADAMTS7 variants and a short LTBP4 fragment were expressed in HEK293(T) cells and purified using anion exchange/gel filtration and anti-flag Ab affinity purification respectively. N-terminal sequencing was outsourced. iTRAQ-TAILS was performed using conditioned medium of fibroblasts co-cultured with HEK cells that expressed either ADAMTS7 or mutated (E389Q) inactive ADAMTS7.

Results
Analysis of recombinant ADAMTS7 by Western blot (WB) identified both latent (+ prodomain) and active (- prodomain) forms, as well as a lower molecular weight (MW) band that was a product of autolysis. Mutagenesis of the active site or prevention of ADAMTS7 activation by furin abolished autolysis. N-terminal sequencing of the autolytic product revealed proteolysis of the Glu732-Ala733 bond in the spacer domain. Mass spectrometry confirmed this and identified a second autolytic cleavage nearby (Glu729-Val730). Mutagenesis of Glu729 and Glu732 to Ala almost completely abolished autolysis, confirming these as major autolytic cleavage sites. These results suggest ADAMTS7 scissile bond specificity favours P1 Glu and small hydrophobic residue at P1'. This information was utilised in the interpretation of iTRAQ TAILS data, leading to the identification of cleavages of Glu-Ala and Glu-Val in latent transforming growth factor β binding proteins, LTBP4 and LTBP3, respectively, in the linker region between the first EGF-like domain and the hybrid domain. These proteins play roles in fibrillin microfibril biology. WB of a recombinant LTBP4 fragment incubated with ADAMTS7 or inactive ADAMTS7(E389Q) confirmed that LTBP4 is susceptible to ADAMTS7 proteolysis. Proteolysis could be inhibited by TIMP3, a known ADAMTS inhibitor, as well as by small molecule metalloprotease inhibitors, Marimastat and Batimastat.

Discussion
We identified Glu-Ala/Val as a scissile bond of ADAMTS7 and LTBP4 as a potential substrate. Further studies are underway to ascertain whether LTBP3/4 are physiological substrates and if so, what the consequences of ADAMTS7 proteolysis are.

P116, CANT1 role in proteoglycan synthesis: an *in vivo* study with a mouse model of Desbuquois dysplasia type 1

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Introduction

Mutations in *CANT1* gene cause Desbuquois dysplasia type 1 (DBQD1) a recessive chondrodysplasia characterized by growth retardation, multiple dislocations and hand deformities. *CANT1* encodes for a calcium activated nucleotidase of the ER/Golgi that preferentially hydrolyses UDP; due to its function and localization a role in proteoglycan (PG) metabolism has been inferred. To investigate this hypothesis and CANT1 role in DBQD we have generated a *Cant1* knock-out mouse (*Cant1*$^{-/-}$ mouse).

Materials and Methods

Morphological analysis of mice were performed by skeletal double staining with alcian blue and alizarin red and by X-ray. Rib chondrocytes were metabolically labelled with ^{35}S-sulfate to analyse PG synthesis and glycosaminoglycan hydrodynamic size. Glycosaminoglycan sulfation was determined by HPLC analysis. PG secretion was studied by pulse-chase ^{35}S-sulfate labelling and morphology of chondrocytes was investigated by TEM. Microarray of ER stress markers was performed on RNA extracted from rib cartilage. Expression level of BiP was confirmed by western blot analysis, while the presence of spliced form of Xbp1 (Xbp1s) was studied by RT-PCR.

Results

Cant1$^{-/-}$ mice were smaller and showed reduced skeletal growth compared with wild-type animals reproducing the growth defects of patients.

In *Cant1*$^{-/-}$ chondrocytes reduced PG synthesis was demonstrated and glycosaminoglycans showed reduced hydrodynamic size and oversulfation compared with wild-types. Pulse-chase experiment demonstrated reduced PG secretion in mutant cells compared with controls and in *Cant1*$^{-/-}$ chondrocytes dilated vacuoles containing electron dense material were observed by TEM. RNA microarray demonstrated no overexpressed ER stress markers in *Cant1*$^{-/-}$ cartilage. Protein level of BiP was normal in *Cant1*$^{-/-}$ cells and Xbp1s was not present in *Cant1*$^{-/-}$ cartilage.

Discussion

Cant1$^{-/-}$ mouse is a useful animal model to study DBQD1 being reminiscent of the patients' phenotype. CANT1 affects proteoglycan synthesis at different levels including glycosaminoglycan chain length, sulfation and secretion in the extracellular matrix. The presence of huge vacuoles in *Cant1*$^{-/-}$ chondrocytes suggests CANT1 involvement in protein retention. However, intercellular retention in mutant cells does not cause ER stress since ER stress markers such as BiP and Xbp1s are normal in *Cant1*$^{-/-}$ chondrocytes and cartilage.

P117, Hyaluronan-binding protein involved in hyaluronan depolymerization (HYBID, alias KIAA1199 or CEMIP) is up-regulated and involved in hyaluronan (HA) degradation in human osteoarthritic cartilage

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Introduction
We showed that HYBID, also called KIAA1199 or CEMIP, plays a key role in degradation of HA in skin and arthritic synovial fibroblasts (PNAS 110:5612-5617, 2013), but its functions in osteoarthritic (OA) cartilage remain elusive. Here, we investigated the expression and roles of HYBID in human OA cartilage.

Materials and Methods
Normal control and OA cartilage samples were obtained from hip joints of the patients with femoral neck fracture and knee and hip joints of OA patients, respectively. They were histologically and immunohistochemically examined. mRNA and protein expression of HYBID was studied by real-time PCR and immunoblotting. Chondrocytes isolated from OA cartilage were stimulated with inflammatory cytokines and growth factors and degrading activity of high molecular weight HA was examined by size-exclusion chromatography. Cellular localization of HYBID and exogenously added HA was observed by confocal microscopy.

Results
HYBID was highly expressed by chondrocytes in the HA-depleted area of OA cartilage, and the HYBID immunoreactivity directly correlated with Mankin score, the histopathological severity of OA lesions of cartilage. Quantitative real-time PCR indicated that the HYBID expression was significantly higher in the OA cartilage than in the control cartilage. In addition, OA chondrocytes exhibited HA-degrading activity, which was abolished by knock-down of HYBID by small interfering RNAs. Although OA chondrocytes also expressed certain levels of hyaluronidase-1, hyaluronidase-2 and CD44, knock-down of these molecules exhibited negligible effects on HA degradation. Double immunostaining of HYBID and clathrin heavy chain revealed that HYBID was localized in the clathrin-coated vesicles and HA was endocytosed within the vesicles in OA chondrocytes. Among eight factors including cytokines and growth factors examined, only tumor necrosis factor-α (TNF-α) stimulated OA chondrocytes to overexpress HYBID.

Discussion
These data are, to the best of our knowledge, the first to demonstrate that HYBID is up-regulated in OA cartilage, and suggest that TNF-α-stimulated HYBID plays a role in HA degradation in OA cartilage.

P118, Hot Topic: Targeting ER stress in Col4a1 mutant mice reduces intracerebral haemorrhaging and highlights tissue specific disease mechanisms

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Introduction

Mutations in the collagen genes *COL4A1* and *COL4A2* cause Mendelian eye, kidney and cerebrovascular disease including intracerebral haemorrhage (ICH), and common collagen IV variants are a risk factor for sporadic ICH. Development of novel treatment for these diseases is urgently needed and requires understanding of pathomolecular disease mechanisms. While mutations cause endoplasmic reticulum (ER) stress and basement membrane (BM) defects, their relative contribut ion to disease and the potential of ER-stress as a therapeutic target remains unclear.

Materials and Methods

Mice were treated with the chemical chaperones, PBA and TUDCA, as a preventative therapy from conception for 5 months or as a 1 month treatment from 4 months of age, when the disease is developed. Phenotype analysis was performed using histopathology, metabolic cage study, slit lamp analysis and magnetic resonance imaging. Electron microscopy, western blotting and immunostaining was performed to determine matrix defects and ER stress levels.

Results

Preventative oral treatment of *Col4a1* mutant mice with PBA and TUDCA reduced sporadic adult ICH and tubular kidney defects. In contrast, glomerular kidney disease and eye disease remained. Interestingly, ICH was also reduced following treatment of adult mice with established disease. PBA acted by reducing ER-stress which was accompanied by increased collagen IV incorporation into basement membranes (BM). However, chemical chaperone treatment did not improve BM structure with a reduced ability of basement membrane to withstand mechanical stress. Comparative analysis between ER stress, matrix defects and pathology revealed that ER stress is associated with vascular and tubular defects, and matrix defects with glomerular disease.

Discussion

Our data provide mechanistic insight into COL4A1 disease, and identify a major role for ER-stress in cerebrovascular disease severity, and matrix defects in eye and kidney defects. Alleviating ER-stress is therefore a valid therapeutic target for treating sporadic ICH and may be feasible using FDA-approved compounds. Future treatments for collagen IV patients will require stratification based on the clinical presentation in patients and mechanism of mutations.

P119, Connecting muscle and matrix: clinical and molecular characterization of six new families with myopathic Ehlers-Danlos syndrome

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Introduction
Collagen XII, encoded by the *COL12A1* gene, is a fibril associated collagen with interrupted triple helices (FACIT) and modulates collagen organization by interacting with fibrillar collagens and extracellular matrix (ECM) proteins. Recently, *COL12A1* mutations were identified in patients displaying a novel overlap syndrome involving muscle and connective tissue, coined 'myopathic Ehlers-Danlos syndrome (mEDS)'. While heterozygous *COL12A1* mutations result in a relatively mild phenotype resembling collagen VI-associated Bethlem myopathy, a homozygous loss-of-function mutation in two siblings results in a more severe congenital mEDS phenotype. The small number of diagnosed patients limits thorough investigation of this newly identified syndrome.

Materials and Methods
We selected a cohort of molecularly unresolved EDS patients with signs of myopathy and sequenced *COL6A1, COL6A2, COL6A3* and *COL12A1*, using next-generation gene panel sequencing.

Results
We identified seven individuals from five families with a heterozygous *COL12A1* mutation and one patient with compound heterozygous *COL6A1* mutations, involving a nonsense and a splice-site mutation resulting in a frameshift. The *COL12A1* mutations comprised one arginine-to-cysteine and four splice-site mutations skipping exon 52, 53, 54 and 56, respectively. Important clinical symptoms included joint hypermobility (n=7/8), hypotonia (n=5/8) and contractures (n=5/8). The phenotype of the *COL6A1*-mutant patient clinically resembled that of the *COL12A1*-mutant patients.
In the ECM of skin fibroblasts of the *COL6A1*-mutant patient, immunocytochemistry revealed a near-complete absence of collagen VI and V. Western blotting showed slightly increased tenascin-X secretion. In *COL12A1*-mutant patient fibroblasts, normal levels of secreted collagens XII, VI and V were observed. Some intracellular accumulation of collagen XII was seen in patients with exon skip 52 and 53, respectively, along with reduced decorin secretion. Secreted tenascin-X was diminished in all *COL12A1*-mutant fibroblasts.

Discussion
In conclusion, we expand the clinical and mutational spectrum of the recently delineated 'myopathic EDS' with seven individuals from five families. We report a novel *COL12A1* arginine-to-cysteine substitution, and four new exon-skipping mutations clustering to the same region, with mutation-dependent abnormalities in the skin ECM. Additionally, we report a patient with a clinically similar mEDS phenotype with compound heterozygous loss-of-function *COL6A1* mutations. Further studies are necessary to understand the pathogenic mechanisms underlying these overlapping phenotypes combining muscle and connective tissue defects.

P120, The role of Latent TGFβ binding protein-4 in elastic fibre assembly and disruption in Urban-Rifkin-Davis syndrome

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Introduction
Urban Rifkin Davis Syndrome (URDS) is a congenital rare disease characterized by high mortality and severely disrupted elastic fibre assembly. URDS is caused by mutations in the *LTBP-4* gene. LTBP-4 is essential for regulating extracellular TGF-□ bioavailability and elastic fibre architecture. Despite the importance of LTBP-4 for intact elastic fibre architecture, the structure of LTBP-4 is not yet defined and the molecular mechanism by which it regulates elastic fibre assembly is poorly understood. Therefore, this study aimed to investigate the nanostructure of LTBP-4 and the impact of URDS point mutations.

Materials and Methods
Wildtype LTBP-4 and URDS mutants were transfected in HEK-EBNA cell line using modified version of pCEP4 vector, expressed, purified and biochemical and biophysical characterization was performed. Wildtype LTBP-4 and URDS mutants were deglycosylated using PNGase F. Wildtype LTBP-4 and URDS mutants were characterized using complementary biophysical techniques including size-exclusion chromatography-multiangle light scattering, sedimentation velocity-analytical ultracentrifugation (SV-AUC), circular dichroism (CD) and small-angle X-ray scattering (SAXS). Surface plasmon resonance (SPR) was performed to investigate the wildtype LTBP-4 and URDS mutants' molecular interaction with other extracellular proteins.

Results
The biochemical analysis showed that the N- and C-terminal regions of LTBP-4 are folded, exist as monomers and have N-linked glycosylation. SV-AUC confirmed the monomeric behaviour for all LTBP-4 constructs but the URD syndrome mutants, Cys244Gly and Cys1156Arg, were aggregation-prone in solution. AUC also suggested an elongated conformation for the LTBP-4 N- and C-termini. CD showed that all LTBP-4 constructs exhibit predominantly □□sheet with random coil conformation, however the Cys1156Arg mutant has a significantly deviated secondary structure *($p < 0.005$)* from the WT and Cys1256Ser mutant structures. SAXS data confirmed the elongated conformation with flexibility for LTBP-4 N- and C-terminal regions and suggested that the Cys1156Arg mutant is wider compared to the WT and Cys1256Ser mutant. SPR confirmed the interaction between LTBP-4 and fibrillin-1. The Cys1156Arg mutation within the TB2 domain significantly perturbed *($p < 0.005$)* this interaction.

Discussion
These data provide novel structural information for LTBP-4 and demonstrate that TB2 domain is important for its folding. The interaction studies demonstrate for the first time that the TB2 domain is involved in the interaction with fibrillin-1.

P121, Cartilage-specific ERp57 knockout mice qualify as a novel model for the analysis of ER stress-related skeletal diseases

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Introduction
Cartilage is essential for skeletal development by endochondral ossification. The chondrocytes produce extracellular matrix (ECM) proteins that undergo folding in the endoplasmic reticulum (ER). ERp57 is essential as it is involved in disulfide bond formation of ECM glycoproteins. If this process fails, ER stress arises and the unfolded protein response (UPR) is induced. ER stress and the UPR in chondrocytes are implicated in the pathogenesis of chondrodysplasias. We generated a cartilage-specific ERp57 knockout mouse (cKO) and analyzed the effects of ER stress during bone development. Additional *in-vitro* analyses in C28I2 cells with CRISPR/Cas9-induced KO of ERp57 (C28/I2-KO) were performed.

Materials and Methods
In WT and cKO littermates bone characteristics (length, structure, growth plate zones, proliferation, apoptosis) were determined by µCT, (immuno)-histochemical and TUNEL staining. Dilated ER cisternae and autophagy were evaluated by TEM. ER stress (Bip, XBP1s, Chop, ATF6, IRE-1) and autophagy marker (LCIII) expression, as well as metabolic activity of chondrocytes (MTT) were investigated after cultivation with ER stress inductor thapsigargin (Tg) and/or the chemical chaperone 4-phenylbutyrate (4-PBA).

Results
Loss of ERp57 triggered ER stress in growth plate chondrocytes of cKO mice and in C28/I2-KO cells. Accumulation of ECM proteins induced the dilation of ER cisternae, reduced proliferation, accelerated apoptosis and resulted in a chondrodysplasia phenotype with shortened long bones. In C28/I2-KO cells, ER stress reduced the metabolic activity and induced autophagy. Increased levels of ER stress marker proteins were detected in WT and KO cells in presence of 0,0001-10µM Thapsigargin. However, 10-50 mM 4-PBA downregulated all ER-stress and autophagy marker proteins investigated.

Discussion
We demonstrated that cartilage-specific loss of ERp57 in mice is sufficient to induce ER stress in chondrocytes resulting in a chondrodysplasia phenotype. This mouse model qualifies to study the role of ER stress and beneficial effects of chemical chaperones, such as 4-PBA, also in degenerative cartilage diseases.

P122, ST: Understanding skeletal disease with cross-species transcriptomics meta-analysis

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Introduction
Skeletal diseases are prevalent in society with a large socio-economic cost and improved molecular understanding is required to develop improved treatments. Results from an increasing number of skeletal transcriptomics experiments provide a new opportunity for mechanistic insight into both fundamental skeletal biology and skeletal disease. However, methods for combined analysis of multiple datasets in a focused disease area are lacking. To address this, we have developed a publicly available online exploration portal, SkeletalVis, for the analysis of skeletal gene expression experiments. This portal will allow exploration and mining across multiple experiments. The integrated analysis of the data will detect similarities between new and existing datasets and identify common features between diseases and experimental models and shared pathogenic mechanisms.

Materials and Methods
A Galaxy pipeline was used to analyse 260 published and available skeletal transcriptomic experiments containing 676 expression responses from ArrayExpress and ENA/SRA in a high-throughput manner to give differentially expressed genes and downstream analysis using standard bioinformatics approaches. The resulting data is available online in an interactive exploration and meta-analysis data portal http://phenome.manchester.ac.uk/

Results
Analysis of the 676 expression response profiles yielded differential expression and downstream analysis include pathways, sub-networks, drug response and transcription factor enrichment. Using related experiments we demonstrate we can recover prior biological knowledge and identify similar experiments with shared biological mechanisms. Using this data portal we identify similarities in expression response in a cross-species group of osteoarthritis animal model expression responses. We further compare recently available osteoarthritis expression data to animal and explant tissue models revealing shared differential gene expression and dysregulated matrix signalling pathways between these models and human diseased tissue.

Discussion
The SkeletalVis provides an online openly accessible data portal for exploration and comparison of skeletal transcriptomic data. We demonstrate its utility in identifying both known and novel relationships between skeletal expression signatures. Importantly, SkeletalVis enables users to upload and analyse data from new experiments and to incorporate them in larger scale meta-analysis. Continued expansion of the repository of data sets is planned as published results become available, which will progressively increase its scale and value as a research community resource.

P123, Investigating drivers of the tumour matrisome, and their role in disease progression

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Introduction

In our recently published work we identified a tumour matrisome composition, that we call the 'Matrix Index', which defines a matrisome that associates with poor prognosis, and is common to many cancer types. Here we present our work to understand how the matrix index is generated, and the interplay between tumour progression, invasion, and tissue remodelling.

Materials and Methods

We have constructed 3D tumour models made from primary human adipocytes, fibroblasts, and mesothelial cells. Tumour cell lines can be introduced either to the 3D gel surface to study metastasis, or implanted into the gel to study late stage disease. These models are useful for investigating matrix remodelling, tumour progression, and invasion.

Results

We find platelets stimulate a matrisome response in malignant cells, which aids proliferation, and invasion of the tumour. Using small molecule inhibitors, we find these matrix index molecules are controlled through the TGFB and hedgehog signalling pathways.

Discussion

Platelet activated malignant cells contributes to the establishment of the matrix index, fibroblast activation, tumour progression, and invasion within our 3D tissue models. Tumour expressed matrix can be suppressed using TGFB receptor and hedgehog signalling inhibitors, which in turn blocks tumour progression. Therefore, blocking tumour matrix production may inhibit tumour progression in combination with clinically used cancer therapeutics.

P124, Regulatory role of collagen XIII in breast cancer

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Breast cancer is significantly influenced by the local microenvironment consisting of different types of cells and the extracellular matrix (ECM). Collagens form a major ECM protein family and are often produced in excess by tumour cells, which leads to disruption of ECM homeostasis and favoring of malignancy. Collagen XIII (ColXIII) belongs to the subfamily of membrane-associated collagens with interrupted triple-helices (MACITs). ColXIII has a short cytosolic domain, and a mainly collagenous ectodomain that can be shed and incorporated into the pericellular matrix. We have previously shown that ColXIII is highly expressed in epithelial and mesenchymal tumours, and it regulates cell adhesion and signaling. The Cancer Genome Atlas (TCGA) analysis suggested that ColXIII is upregulated in solid tumours. In the current study, we have explored the expression and specific roles of ColXIII particularly in breast cancer. Bioinformatic analysis showed that high ColXIII expression associates significantly with the poor survival particularly in estrogen receptor (ER) negative patients. Immunohistochemical stainings of human breast cancer tissues showed that ColXIII is abundantly expressed in tumour cells. In the MMTV-PyMT mouse mammary carcinoma model, lack of *Col13a1* gene lead to significantly reduced tumour cell proliferation and tumour growth in a later stage of carcinogenesis in comparison with the control PyMT mice. Moreover, ColXIII expression was highly upregulated in the triple negative human breast cancer cell lines and siRNA-mediated knockdown of ColXIII significantly reduces the proliferation and viability of these cells. Hence, our data show that ColXIII is actively involved in the breast tumourigenesis. Further studies will be conducted to identify the underlying molecular mechanisms through which ColXIII regulates the breast cancer progression.

P125, Investigation of the functional effects of SDC4 cleavages by MT4-MMP in the EGFR signaling pathways

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Introduction
The high expression level of EGF receptor (EGFR) in most of triple negative breast carcinomas (TNBC) provides an option for a potential targeted therapy. Interestingly, we recently discovered that MT4-MMP, a membrane-type matrix metalloprotease expressed at the surface of breast cancer cells colocalizes and interacts with EGFR in TNBC. These recent findings led to the novel concept that MT4-MMP contributes to EGFR pathway regulation. The main goal here is to investigate in-depth the molecular mechanisms underlying the dual function of MT4-MMP, namely 1) its impact on the EGFR pathway activation, which does not rely on its proteolytic function and 2) its capacity to exert pro-angiogenic and pro-metastatic effects through its proteolytic activity.

Results
Our first aim is to determine the domains of MT4-MMP that interact with EGFR. With this aim, we have designed different mutated and truncated forms of MT4-MMP that were transfected into Cos-1 cells. The hemopexin domain of MT4-MMP is sufficient to induce EGFR/MT4-MMP interaction and to promote EGFR phosphorylation.
With the aim to search for MT4- MMP substrates, we have used the I-TRAQ technology and identified syndecan-4 (SDC4) as a cell membrane substrate. The presence of EGFR/SDC4/MT4-MMP in a molecular complexe was assessed by immunostainings and co-immunoprecipitation assays. FACS analyses showed an increased cleavage of syndecan-4 when MT4-MMP was overexpressed. In functional assays, we are providing evidence that SDC4 cleavage by MT4MMP promotes the migration of cells overexpressing MT4MMP. SDC4 silencing through siRNA reduced this migratory effects and in opposite, the addition of soluble SDC4 ectodomain in control cells exerted a pro-migratory effect in MDAMB231 cells.

Discussion
These data provide evidence for an unexpected crosstalk between MT4-MMP, SDC4 and the EGFR. These results give new insights into a new pro-migratory effect of MT4-MMP through its proteolytic activities that might have implication on EGFR inhibition strategies for EGFR positive cancers.

P126, ST: New Zebrafish models for recessive osteogenesis imperfecta

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Introduction
Osteogenesis imperfecta (OI) is a heritable disorder characterized by bone deformity, skeletal fragility and short stature. Cartilage associated protein (CRTAP), proline 3-Hydroxylase 1 (P3H1) and Cyclophylin B (PPIB) are components of the ER-resident complex, involved in the hydroxylation of specific proline residues in collagen type I α chains. Mutations in these proteins are responsible for recessive OI type VII, VIII and IX, respectively. Murine models for these diseases exist, but the availability of zebrafish models will allow a deeper understanding of the phenotype at early developmental stage as well as will favour drug screening with multiple molecules reducing amount, timing and cost. We applied CRISPR/Cas9 editing strategy to generate knock out models for Crtap and P3h1 and performed their characterization.

Materials and Methods
Syntheny analysis was performed using Genomycus v87.01. CHOPCHOP was used for guide RNA (gRNA) selection. pT7gRNA vector was used for gRNA subcloning and *in vitro* transcription and the pT3TS-nCas9n plasmid for Cas9 mRNA synthesis. Morphometric measurements of mutants were performed using Leica LAS v4.5 software. Alizarin red staining and x-Rays were used for bone characterization. Mutant collagen type I was characterized by SDS-PAGE. The growth rate of caudal fin was performed measuring length of fin rays after amputation.

Results
*p3h*1 and *crtap* are highly conserved between tetrapods and teleosts. We successfully generated the knock out for both genes in zebrafish using CRISPR/Cas9. P3h1 and Crtap homozygous mutants are smaller than WT and show a delayed mineralisation as revealed by alizarin red staining. Their phenotype is worsening with age and adult mutant fish are characterized by vertebrae disorganization and skeletal deformity, as observed by x-Rays and µCT scans. Collagen type I has abnormal electrophoretic migration. Bone formation, evaluated on fin regeneration, was delayed in *p3h1* mutants compared to WT.

Discussion
We proved the goodness of zebrafish model to reproduce the phenotype of the recessive OI type VII and VIII. Our goal will be to use our models for drug screening in order to pave the way to new pharmacological treatments.

P127, ST: *SLC10A7* mutations in human and mouse cause a skeletal dysplasia with amelogenesis imperfecta mediated by GAG biosynthesis defects.

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Introduction

Skeletal dysplasias with multiple dislocations are a group of severe disorders characterized by dislocations of large joints, scoliosis, short stature and a variable combination of cleft palate, heart defects, intellectual disability and obesity. With the help of massively parallel sequencing technologies, the majority of these rare disorders have been linked to pathogenic variants in genes encoding glycosyltransferases ("linkeropathies"), sulfotransferases, epimerases or transporters, required for glycosaminoglycan (GAG) biosynthesis. These findings support the existence of a new group of inborn errors of development defined by impaired GAG biosynthesis. However, several findings suggest that GAG synthesis is more complex than previously described and that there are a number of partners of unknown function still to be identified. Especially, correct GAG biosynthesis is dependent on a tightly regulated Golgi pH and ion homeostasis.

Results

Using exome sequencing, we identified homozygous mutations in *SLC10A7* in six individuals with a skeletal dysplasia with dislocations and amelogenesis imperfecta. Common features were severe growth retardation <-3SD, cleft palate, yellow/brown teeth, knee dislocations, spine anomalies and advanced carpal ossification. *SLC10A7* encodes a 10-transmembrane-domain transporter located at the plasma membrane, with a yet unidentified substrate. Functional studies *in vitro* demonstrated that *SLC10A7* mutations were loss-of-functions mutations reducing SLC10A7 protein expression. We generated a *Slc10a7*$^{-/-}$ mouse model which displayed short long bones, growth plate disorganization and tooth enamel anomalies, recapitulating the human phenotype. Furthermore, we identified decreased heparan sulfate levels in *Slc10a7*$^{-/-}$ mouse cartilage and patient fibroblasts. Finally, we found an abnormal N-glycoprotein electrophoretic profile in patient blood samples.

Discussion

The link of SLC10A7 with Golgi divalent ion homeostasis is not clear but suggested by yeast studies showing that SLC10A7 orthologs could act as negative regulator of cytosolic calcium homeostasis. Together, our findings support the involvement of SLC10A7 in glycosaminoglycan synthesis and specifically in skeletal and tooth development.

P128, Substrate Specificity of Collagen Prolyl 4-Hydroxylases

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Introduction
Collagen prolyl 4-hydroxylases (C-P4H) catalyze the formation of 4-hydroxyproline in collagenous -XPG- sequences. Enzyme tetramers consist of two identical catalytic alpha subunits and protein disulphide isomerases as a beta subunits. P4HA1, P4HA2 and P4HA3 encode the alpha subunit genes. Mutations in P4HA1 have been described in congenital disorder manifesting as early-onset joint hypermobility, joint contractures, muscle weakness, bone dysplasia and high myopia. Mutations in P4HA2 cause myopia. In this work, we show collagen sequence specificity of P4H isoforms.

Materials and Methods
We used prolyl 4-hydroxylase mutant mouse (P4ha2 +/-;P4ha2+/-, P4ha2-/-; P4ha1+/-;P4ha2-/-) skin and P4ha1-/- embryonic fibroblasts to extract collagen for tandem mass spectrometry in order to identify proline residues that have affected hydroxylation. Results were confirmed by activity assay using (XPG)5 peptides with different X-position amino acids and purified recombinant C-P4H enzymes.

Results
Extracted collagen was analysed by mass spectrometry. Results were confirmed by in vitro activity assays. Data indicated that deletion of P4ha1 leads to substantial decrease in 4-hydroxylation of prolines. Deletion affects many different hydroxylation sites. Data showed also that C-P4H-I is very poor in catalyzing hydroxylation of prolines that follow aspartate or glutamate, whereas C-P4H-II can efficiently hydroxylate those. In addition, absence of C-P4H-II leads to slight underhydroxylation when X-position amino acid is uncharged serine, glutamine or threonine. In contrast, deletion leads to slight overhydroxylation when X-position amino acid is aliphatic. In conclusion, we noticed a role of X-position amino acid to adjacent proline to be hydroxylated.

Discussion
Our results indicate that multiple isoforms of C-P4H are needed as single isoform cannot fully hydroxylate collagen. We observed clear sequence specific prolyl 4-hydroxylation by different isoenzymes. Data explains some of the phenotypes observed in mouse models and human prolyl 4-hydroxylase related diseases. Data suggests that catalytic domain of C-P4H has selectivity in hydroxylation sites.

P129, Proteolysis of Versican and COMP by ADAMTS-4, -5 and -7

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Introduction
ADAMTS-4 and -5 cleave structurally important extracellular matrix (ECM) proteoglycans, such as versican. This proteoglycan mediates a pivotal role during development and in cardiovascular disease. ADAMTS-4 has also been reported to cleave a non-proteoglycan molecule, Cartilage Oligomeric Matrix Protein (COMP), previously identified as a substrate for another ADAMTS family member, ADAMTS-7. However, little is known about how these enzymes recognise, bind and cleave their substrates, nor if any difference exists between proteoglycan and non-proteoglycan substrates. To better understand the mechanisms involved, we have determined the kinetic parameters, k_{cat} and K_m, of versican cleavage and the importance of individual ADAMTS-4 and -5 domains in the proteolysis of versican and COMP.

Materials & Methods:
Recombinant ADAMTS-4, -5 and -7 variants, versican and COMP were expressed and purified. Proteolysis of versican was analysed using a sandwich ELISA which specifically measures cleavage at the Glu441-Ala442 bond. Proteolysis of COMP was analysed by semi-quantitative Western Blotting.

Results:
Analysis of versican cleavage by ADAMTS-5 revealed a ~15-fold higher catalytic efficiency compared to ADAMTS-4 (k_{cat}/K_m =2.6 x 10^6 vs 1.7 x 10^5 M^{-1} sec^{-1}). Interestingly, this was mediated by a >200-fold higher turnover number (k_{cat}), which compensated for a ~20-fold increase in the Michaelis-Menten constant (K_m). A comparison of COMP cleavage by ADAMTS-4, -5 and -7 showed that only ADAMTS-4 could efficiently cleave COMP at physiological (low nM) enzyme concentrations. Deletion of the ADAMTS-4 spacer domain severely reduced proteolysis of versican (90%) and COMP (70%). In contrast, deletion of both the spacer and cysteine-rich domains in ADAMTS-5 was necessary for a similar reduction (50%) in versican cleavage, suggesting a difference in the involvement of individual domains in substrate recognition and binding.

Discussion
For the first time, we have determined the kinetic parameters of versicanase activity for ADAMTS-4 and -5. Our results provide a mechanistic rationale for a more potent versicanase activity of ADAMTS-5 than ADAMTS-4. However, under conditions where its expression is upregulated (i.e. its concentration is increased), such as inflammation, ADAMTS-4 may be more disruptive for the ECM integrity, due to its high affinity (i.e. 20-fold lower K_m) for versican and higher COMPase activity.

P130, Using patient derived induced pluripotent stem cells to model multiple epiphyseal dysplasia

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Introduction
Multiple epiphyseal dysplasia (MED) is a chondrodysplasia characterised by delayed epiphyseal endochondral ossification, resulting in disproportionate short stature and early onset osteoarthritis. MED can be caused by heterozygous mutations in COMP, MATN3, COL9A1, COL9A2 and COL9A3, or bi-allelic mutations in SLC26A2. Human induced pluripotent stem cells (hiPSCs) are reprogrammed somatic cells which can differentiate to form all body tissues and have excellent potential for tissue regeneration as well as providing models of human disease. Our aim is to generate an *in vitro* hiPSC model of growth-plate development in order to better understand MED.

Materials and Methods
HiPSCs were generated from peripheral blood mononuclear cells (PBMCs) of 3 related MED individuals who are heterozygous for a MATN3 p.Val194Asp mutation (V194D) and 4 healthy controls. HiPSC were differentiated to growth-plate-like chondrocytes via an iPSC-MSC-like intermediate, followed by TGFβ3 + BMP2 induced chondrogenic pellet culture for 21 days.

Results
Healthy and V194D hiPSCs were able to differentiate to iPSC-MSCs which displayed typical MSC morphology, expressed MSC markers (CD90, CD105, CD44 and CD73) and were capable of generating cartilage and bone. After 21 days in TGFβ3 + BMP2-containing medium V194D chondrogenic pellets were significantly larger in size, stained more strongly for cartilage associated sulphated glycosaminoglycans (Alcian blue and Safranin O), and expressed significantly higher levels of SOX9, COL2A1 and ACAN transcript. RNA-Seq validated these differences and pathway analysis of V194D vs healthy identified enrichment of terms such as ‘dwarfism’ and ‘skeletal system development’. Immunohistochemistry and confocal co-localisation analysis showed matrilin-3 was retained within the ER of the V194D mutant pellets, interestingly we observed little evidence of ER stress.

Discussion
These data suggest V194D mutant cells respond differently during TGFβ3 + BMP2 induced chondrogenesis, which may be caused by altered matrix regulation of growth factor availability. This model provides novel insight into MED disease pathogenesis and will enable screening of pharmaceutical products.

P131, Structure and function of collagen VI in health and myopathy

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Introduction
Collagen VI is an integral extracellular matrix (ECM) protein involved in key functions of skeletal muscle. Disruption to the assembly or export of Collagen VI microfibrils results in the conditions of Bethlem Myopathy (BM) and Ullrich Congenital Muscular Dystrophy (UCMD). Collagen VI is composed of three chains α1, α2 and α3, largely made up of protein protein interaction subunits, termed von Willebrand factor Type A (VWA) domains. All three chains can contain mutations in their triple helical (TH) regions and globular VWA domains which lead to a disease phenotype. Here, we functionally characterize a UCMD cohort with a COL6A1 TH pseudoexon, and structurally characterize mutation prone VWA domains of the collagen VI α3 chain.

Materials and Methods
Structural studies: Recombinant protein expression, X-ray crystallography, small angle X ray scattering (SAXS). *Mutation studies:* Western blot of patient material, immunogold electron microscopy (EM), mass spectrometry (MS).

Results
Structural studies: Crystals for the α3 chain N2 domain were harvested and diffracted to 1.8Å resolution. SAXS shows tandem construct α3 chain N4-N5 in rigid conformation. *Mutation studies:* Secretion of non-triple helical pseudoexon containing α1 chain into ECM, EM shows this chain forms clusters in the ECM, MS highlights change in collagen VI binding partners in patient material.

Discussion
Structural studies:
The tandem construct α3N4-N5 SAXS model reveals a spring like conformation of tandem VWA domains, suggesting a mechanical role for the extended N-terminal in normal physiology. The α3N2 domain has been shown to harbor several mutations found in BM/UCMD patients. Mapping of such mutations onto this structure reveals possible pathogenic mechanisms according to their locations, buried deep within the structure on a central β sheet resulting in misfolding and ER stress, or on the surface of the domain, likely disrupting intra/intermolecular interactions.
Mutation studies: Patient dermal fibroblasts secrete non-triple helical pseudoexonic α1 chain into the ECM, which is not incorporated into the deposited collagen VI matrix. The secretion profile and extreme severity of the phenotype highlights a novel extracellular “gain of misfunction” toxic disease mechanism, manifesting perhaps through aberrant self-interaction with unaffected collagen VI microfibrils or sequestration of collagen VI binding partners.

P132, Isolation, culture and characterization of cells from human abdominal aortic aneurysm

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Introduction
Abdominal aortic aneurysm (AAA) is common and asymptomatic life - threatening illness. It comes into existence as a result of abdominal aortic walls dissection, which leads to blood vessel rupture and patient's death in great measure. AAA is a multifactorial clinically heterogenic disease, therefore, an indication of one main factor causing the aneurysm is difficult.

Materials and Methods
The aim of the study was to standardize the cell culture methods for cells obtained from AAA specimens and determination of their phenotypes. The cells were isolated from separated inner, external and median layer of each AAA fragment. The cells were compared to control commercial cell types from health human abdominal aortas. Isolated cells were cultured in an appropriate standard culture media. Subsequently, the cell cultures were analysed by flow cytometry, immunochemistry and macroscopic methods.

Results
Cells from the inner layers of aorta of all patients needed the shortest time to reach primary confluence. The cells from external layers of all patients presented the longest time to reach primary confluence. The shortest time to reach confluence after the recovery from the banking in liquid nitrogen presented the cells from the middle layers of all patients. In all the layers only small fractions of cells presented layer specific cell type markers. Majority of the cells from every layer were the cells positive for CD90 considered as fibroblast marker. The cells from the layers did not show the expected morphology, such as for endothelium in the inner layer, smooth muscle in the middle and fibroblasts in the external layer.

Discussion
Abdominal aortic aneurysm is a very important life-threatening health problem. Therefore, finding a specific prognostic marker, which is associated with the presence and progression of AAA is urgently crucial. The studies here have confirmed that in the wall of the AAA there is a lack of specific cell type composition in comparison to the normal abdominal aorta wall. Further studies are planned on the cells molecular pathobiology in AAA as well as the cross-talk between the ECM and the cells in AAA.

P133, Towards an atlas of matrisome regulation in cancer

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Introduction
Alterations in the quantitative and qualitative content of the tumor microenvironment are of primary importance in the neoplastic process, so much so as to have been recognized since the pioneering theory of the "bad seed in bad soil" formulated by Paget in 1889. Nowadays, the complex network of extracellular matrix (ECM) components, growth factors, cytokines and enzymes is better recognized as a whole "omic" world (defined as the "matrisome"), regulated by an intricated and yet poorly understood network of transcriptional programs. Based on the enormous importance of matrisome regulation in cancer, we are developing a "first-of-it-kind" integrated approach to: 1) catalogue mutational and variational events in the cancer matrisome, 2) define which matrisome genes are specific to any given tumor and, 3) unravel transcriptional regulatory pathways operating on matrisome genes.

Materials and Methods
Complete mutational, variational, transcriptional and clinical data from The Cancer Genome Atlas (TCGA) PANCAN cohort were downloaded, filter for quality and completeness and used for further analyses, for a total of 822 matrisome genes assessed in 10487 patient samples representing 32 human tumors. Various profiling and clustering algorithms, together with a custom-made gene regulatory network (GRN)-inferring software, were used for the analyses.

Results
Different tumors vary notably in the amount of mutations and variations per each given class of matrisome genes, but also show tissue- and system-of-origin similarities for what concerns the expression of these genes, so much so that clusters of neoplasms (with important differences in their clinicopathological features) can be inferred. Furthermore, common GRNs seem to characterize different neoplasms belonging to the same cluster, evidencing a relatively small amount of master transcriptional programs which similarly regulate matrisome genes in different cancers.

Discussion
Our results represent, to date, the most complete effort at characterizing and understanding the molecular biology of cancer matrisome, and offer new insights into its regulatory mechanisms with significant consequences for clinical and pharmacological research.

P134 Withdrawn

P135, Intervention of the crosstalk between collagen XVIII and ErbB receptors augments therapeutic outcome in breast cancer

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In spite of evolving treatments breast cancer is still the major cause of cancer deaths among women. Current precision medicine approaches that target growth factor receptors are efficient options to treat advanced breast cancers. However, these approaches exclude the context of microenvironment, in which cancer cells adhere, survive and multiply. Deregulated extracellular matrix (ECM) dynamics is one the hallmarks of cancer and has critical influence in cancer progression and metastasis. In the current study, we investigated the expression and roles of collagen XVIII (ColXVIII), a ubiquitous basement membrane (BM) component, in breast cancer and evaluated its clinical and translational potential. We found that high ColXVIII expression is significantly associated with high tumour grades, poor relapse and metastasis-free survival in HER2 and triple negative breast cancer (TNBC) subtypes. While in normal breast tissue and in ductal carcinoma *in situ* ColXVIII expression is restricted to epithelial and vascular BMs, it is upregulated in tumour cells of invasive ductal carcinoma. In addition, ColXVIII often co-localizes with epidermal growth factor receptors EGFR and HER2, which are commonly upregulated in advanced breast cancer types. Genetic intervention of ColXVIII in human breast cancer cells reduces cell proliferation, and in the MMTV-PyMT mouse model halts cancer progression and lung metastasis. Moreover, ColXVIII and EGFR co-precipitate in the same protein complex suggesting a role for this collagen in regulating the crosstalk between EGFR and ECM in breast cancer. Interestingly, inhibition of ColXVIII augments the efficacy of EGFR/HER2 targeting therapies and favors to overcome drug resistance. In summary, ColXVIII has a key regulatory role in breast cancer and its intervention opens up novel therapeutic options for treatment of advanced breast cancers.

P136, Exposure of tropoelastin to the inflammation generated oxidant peroxynitrous acid, gives high yields of nitrated tyrosine residues, di-tyrosine cross-links and altered protein structure and function

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Introduction
Elastin is an abundant extracellular matrix protein in elastic tissues, including the lungs, skin and arteries, and comprises 30-57 % of the aorta by dry mass. Monomeric tropoelastin (TE), undergoes complex processing during elastogenesis to form mature elastic fibers. Peroxynitrous acid (ONOOH), a potent oxidizing and nitrating agent, is formed *in vivo* from superoxide and nitric oxide radicals. Considerable evidence supports ONOOH formation in the inflamed artery wall, and a role for this species in the development of human atherosclerotic lesions, with modified extracellular matrix implicated in lesion development and rupture. Hypothesis: The unusual amino acid composition and structure of tropoelastin would make this protein highly susceptible to modification and structural alteration, with consequences for protein function.

Results
TE is highly sensitive to ONOOH, with extensive dimerization, fragmentation and nitration of Tyr residues to give 3-nitrotyrosine (3-nitroTyr) detected, by SDS-PAGE with silver staining or western blotting, with equimolar or greater levels of oxidant. Damage increased in an oxidant dose-dependent manner. Quantification of Tyr loss and 3-nitroTyr formation, by UPLC and amino acid analysis, indicates extensive Tyr modification with up to two modified Tyr per protein molecule, and up to 8% conversion of ONOOH to 3-nitroTyr. These effects were modulated by bicarbonate, a competitive target for ONOOH. Using mass spectrometry (MS) peptide mapping, 3-nitroTyr formation was detected at 12 of 15 Tyr sites in TE treated with equimolar or higher levels of ONOOH. Label-free quantification revealed extensive nitration (>50% modification) at several sites with high oxidant (250-fold) excesses. Inter- and intra-protein di-tyrosine cross-links were characterized by MS. TE treatment with ONOOH lowered both the concentration at which TE coacervates, and increased the rate of this process. Studies on human atherosclerotic lesions using immunohistochemistry showed colocalization of 3-nitroTyr with elastin epitopes, consistent with modifications *in vivo*, and also an association of 3-nitroTyr containing proteins and elastin with lipid deposits.

Discussion
These data suggest that exposure of TE to ONOOH gives marked chemical, structural and functional changes to TE and altered matrix assembly, and that such damage accumulates in human arterial tissue during the development of atherosclerosis.

P137, Generation and characterization of an animal model of chondrodysplasia with joint dislocations gPAPP type

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Introduction
Chondrodysplasia with joint dislocations gPAPP type is a recessive skeletal disorder caused by mutations in the *IMPAD1* gene encoding for gPAPP, a Golgi-resident 5'-phosphoadenosine 3'-phosphate (PAP) 3'-phosphatase that hydrolyzes PAP, the by-product of sulfotransferase reactions, to AMP and phosphate. This enzyme is involved in glycosaminoglycan (GAG) sulfation and its function has been confirmed in an Impad1 knockout mice (PNAS, 105, 11605-12). Mutant mice are lethal at birth precluding the study of IMPAD1 in post-natal skeletal development which is important in chondrodysplasia gPAPP type because patients do not have a lethal phenotype. For this reason we have generated a conditional knock-in mouse that reproduces a patient's missense mutation.

Materials and Methods
Morphological observations were performed by X-rays and skeletal staining with alcian blue and alizarin red. RNA was extracted from skin of newborn mice and *Impad1* expression was evaluated by qPCR. Cartilage proteoglycan sulfation analysis was performed by HPLC disaccharide analysis.

Results
The first Impad1 conditional knock-in for a missense mutation (Asp175Asn) reported in a patient was generated. Unexpectedly, the phenotype of mutant homozygous mice was lethal at birth with severe hypoplasia of the skeleton. Biochemical analysis of cartilage from wild-type, knock-in and knock-out mice demonstrated that the amount of non sulfated disaccharide relative to the total amount of disaccharides was significantly increased in knock-in mouse, equally to the knockout mouse. Since the Knock-in mouse shows the same phenotype as the Knock-out we analyzed the molecular basis of the lethal phenotype. Potential alternative splicing of the targeted allele were investigated by RT-PCR: in the wild-type a transcript corresponding to the whole coding sequence was detected, while in the homozygous mutants two different Impad1 transcripts lacking exon 2 or exons 2-3 were observed.

Discussion
Results demonstrated that the mouse lethality was due to alternative splicing of the targeted allele caused by the strategy used in the set-up of the conditional knock-in targeting vector. For this reason, a new knock-in mouse strain has been generated using a different gene targeting vector and is currently under investigation.
Work supported by Telethon-Italy (grant GEP15062) and the European Community (FP7, "Sybil" project, grant 602300).

P138, Mutations in the COL1A1 and COL1A2 genes associated with osteogenesis imperfecta (OI) types I or III

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Introduction
Osteogenesis imperfecta (*OI*) is a heterogenic, connective tissue and extracellular matrix related disorder, manifested by bone fragility, with autosomal dominant, rarely recessive, inheritance and $1/10^4$ to $1/3 \times 10^4$ prevalence. Although over 85% of *osteogenesis imperfecta* (*OI*) cases are associated with mutations in the procollagen type I genes (*COL1A1* or *COL1A2*), no hot spots for the mutations were associated with particular clinical phenotypes.

Materials and Methods
Eight patients that were studied here, diagnosed with *OI* by clinical standards, are from the Polish population with no ethnic background indicated. DNA for analysis was isolated from blood samples and was PCR amplified followed by DNA sequencing on ABIPrism3130xl sequencer.

Results
Previously unpublished mutations were found in six out of those eight patients. Genotypes for polymorphisms (Sp1 – rs1800012 and PvuII – rs412777), linked to bone formation and metabolism were determined. Mutations were found in exons 2, 22, 50 and in introns 13 and 51 of the *COL1A1* gene. In *COL1A2*, one mutation was identified in exon 22.

Discussion
Mutation locations analysed here did not point directly to type of *OI*. The resulting changes in the encoded amino acids are not sufficient for predicting how severe the disorder will be. The consistence of the glycine codon changes to the cysteine codon (at position 403 in patient 19/F) correlates with commonly accepted Gly to Xxx substitutions resulting in a more severe type III or a lethal type II of *OI*. In addition, the possible exon skipping effect of I51 mutation at the 4248+1 position could explain the more severe type III phenotype manifested by a tremendous number of bone fractures. Results obtained by detection of mutations in genes encoding procollagen type I in patients diagnosed with either type I or III *OI* revealed that the causative mutations might occur anywhere in both genes.

P139, ST: Ablation of epidermal collagen chaperoning by Hsp47 results in dermal fibrosis

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Introduction
Heat-shock protein 47 (Hsp47), an essential chaperone during procollagen biosynthesis, is responsible for the stabilization of pro-collagen and its secretion into the extracellular matrix. Mutational inactivation in humans or genetic ablation in mice showed that Hsp47 is indispensable for bone and cartilage formation during development, but also specific ablation in the dermis leads to embryonic lethality. Hsp47 is described to principally interact with fibrillar collagens; however, it is not fully investigated whether also other members of the collagen superfamily such as transmembrane collagens require Hsp47 assistance for triple-helix stabilization and proper network assembly. To explore a role of Hsp47 on epidermal transmembrane collagen chaperoning *in vivo* we generated epidermal specific Hsp47 knock-out (Hsp47 eKO) mice.

Materials and Methods
K14 Cre/loxP system; electron microscopy; second harmonic generation microscopy; mass-spectrometry; western-blot analysis; collagen-crosslink analysis; confocal immunofluorescence microscopy, ELISA-style binding assay

Results
The keratinocyte-specific genetic ablation of Hsp47 in mice results in severe dermal fibrosis characterized by altered dermal collagen crosslinking and fibril formation. Isolated Hsp47-deficient basal keratinocytes revealed significant reduction of collagen XVII expression and shedding which was accompanied by increased secretion of TGF-β. ELISA-style binding assays identified collagen XVII as new interaction partner for Hsp47.

Discussion
Our data show that Hsp47 interacts with members of the MACIT (Membrane Associated Collagens with Interrupted Triple helices) collagen subfamily such as collagen XVII. Our finding that epidermal ablation of Hsp47 leads to excessive dermal fibrosis suggests a so far unknown role of Hsp47 in epidermal-dermal cross-talk.

P140, Characterization of Bone Extracellular Matrix Produced By Recql4-Deficient Osteoblasts

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Introduction
Bone is a complex and mineralized tissue under permanent remodeling, in which the main cellular actors are osteoblasts (OB), synthesizing the mineral matrix, osteoclasts (OCL), in charge of bone resorption, and osteocytes (OST), acting as mechanosensors. The bone extracellular matrix (ECM) is a dynamic network of molecules secreted by OB and OST which in turn regulate the behavior of all bone cells by modulating their proliferation, differentiation and function. As an example, senescent OB create a defective microenvironment through an altered secretome, which in turn stimulates OCL function. This observation highlights the connection between OB secretome, ECM and bone cells behavior. Our laboratory is studying the *recql4*$^{-/-}$ murine model potentially associated with altered OB secretome. RECQL4 is a DNA helicase involved in genomic stability and its dysfunction has been associated with cellular senescence. Aiming to decipher the mechanisms underlying bone loss in our model, we started investigating the interactions between the OB-produced mineralized ECM and bone cells.

Materials and Methods
The femur microarchitecture from *recql4*$^{-/-}$ and *recql4*$^{+/-}$ control mice was analyzed by micro-Computed Tomography. Primary OB were isolated from those mice and used to synthesize bone ECM *in vitro* which were examined for protein and mineral composition as well as for ultrastructure using different approaches such as calcium staining, proteomic analysis and scanning electron microscopy.

Results
recql4$^{-/-}$ mice exhibit a premature bone aging phenotype. Bone matrices produced by *recql4*$^{-/-}$ OB tend to be less mineralized than those from control OB. A first set of proteomic analyses revealed 3 proteins missing from the mutant ECM and known to be involved in osteogenesis regulation: one that seems to be required for matrix mineralization by OB; another, that is involved in osteoblastogenesis regulation and a coupling factor linking bone resorption and bone formation.

Discussion
Depletion of any of the 3 candidate proteins we have identified might lead to a phenotype observed in our mouse model. Experiments to confirm these findings are in progress. The subsequent step will be to compare differentiation and function of osteoclasts on mineralized matrices synthesized by *recql4*$^{-/-}$ and *recql4*$^{+/-}$ OB.

P141, Keratoconus: New insights in the underlying pathomechanism by hTNFtg and syndecan-4 deficient mice

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Introduction
The corneal stroma consists of orthogonally stacked lamellae formed by thin collagen fibrils evenly spaced in parallel. Among other requirements, activity of lysyl oxidase (LOX) and tissue transglutaminase (TG2) is essential for lamellar stacking and thin fibrils, respectively. However, the mechanism for development of this suprastructure is still unknown. Recent studies suggest that loss of syndecan-4 has regulatory effects on cross-linking enzymes, and thereby, resulting in structural ECM changes. Interestingly, in the human eye disease keratoconus (KC), structural and compositional changes leads to disruptions of the lamellar organization with thinning and scarring of the central part of the cornea but pathomechanisms are unknown. Both genetic and environmental factors have been associated with KC and recent studies suggest that, at least in part, inflammatory conditions (e.g. high TNF-alpha levels) might play a role in KC.

Materials and Methods
We have studied the suprastructural organization of the corneal stroma of hTNFtg and syndecan-4 deficient mice as well as of KC patients by TEM. Disruptions of collagen structures were visualized by collagen hybridizing peptides B-CHP. Moreover, 3D-cell cultures of isolated keratocytes were analyzed by TEM and for TG-activity.

Results
Sheets of orthogonally arranged collagen fibrils were found in the stroma of wild-type mice as well as of human controls. However, lamellae were disrupted in hTNFtg as well as in syndecan-4 deficient mice. Interestingly, however, a similar morphology of the stroma was found in KC patients. Moreover, keratocytes revealed evidences for apoptosis and collagen fibrils formed were thicker with a visible banding pattern. 3D-cell cultures of human KC keratocytes generated an altered ECM with reduced TG-activity in comparison to controls. Moreover, binding of B-CHP was stronger in KC samples as well as in hTNFtg mice.

Discussion
The disruption of the lamellar organization of collagen fibrils in hTNFtg and syndecan-4 deficient mice is similar to that found in corneal stroma of KC patients. Thus, cross-linking as well as inflammatory factors could be crucial factors for manifestation of KC supported by more degraded collagens visualized by a higher binding of B-CHP. On the other hand, analysis of syndecan-4 deficient corneas will provide new insights in mechanisms of lamellae formation and fibril diameter control, respectively.

P142, Mutations in the collagen I prolyl 3-hydroxylation complex cause altered cellular homeostasis

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Introduction
The brittle bone disease osteogenesis imperfecta (OI) is a collagen-related disorder associated to dominant, recessive or X-linked transmission. Among the recessive forms, OI types VII, VIII, and IX are due to mutations in *CRTAP*, *P3H1*, and *PPIB* genes, respectively, coding for the components of the endoplasmic reticulum (ER) complex that catalyses 3-hydroxylation of type I collagen α1(I)Pro986. In dominant OI, due to mutations in type I collagen genes, we recently demonstrated the presence of an altered cytoskeleton and of an ER retention of overmodified collagen, causing cellular stress that contributes to OI pathogenesis. Here we investigated the effect of mutations in the *CRTAP*, *P3H1*, and *PPIB* genes on cellular homeostasis in primary fibroblasts from OI patients.

Materials and Methods
Cytoskeleton and nucleoskeleton asset were investigated by functional proteomic and confocal analyses. ^{3}H proline labelled type I collagen was analysed by electrophoresis. ER morphology was evaluated by transmission electron microscopy. The activation of unfolded protein response (UPR), autophagy and apoptosis were determined by western blot and FACS. Cells were treated with 4-phenylbutyrate (4-PBA) and the effect was evaluated by western blotting. General protein secretion was determined by labelling with ^{35}S-L-methionine and ^{35}S-L-cysteine.

Results
Altered expression/distribution of lamin A/C and cofilin-1 revealed an aberrant organization of nucleus and cytoskeleton in mutant fibroblasts. The overmodified collagen, synthesized by OI patients cells and partially intracellular retained, caused ER enlargement and the activation of the PERK branch of the UPR leading to an increased cell death. The treatment with 4-PBA reduced UPR, ER cisternae size and apoptosis thanks to its stimulatory effect on general protein secretion and autophagy.

Discussion
We have shown that recessive OI forms with mutations in the collagen I prolyl 3-hydroxylation complex share common features with classical dominant OI, such as cytoskeleton alteration and cellular response to altered collagen molecules. Furthermore, in both cases, 4-PBA rescued the altered homeostasis caused by the presence of overmodified collagen I, demonstrating that cellular ability to cope with stress can be a valid target for OI treatment.

P143, Bone-specific abnormal collagen post-translational chemistry and cross-linking causing bone fragility in Bruck Syndrome caused by compound heterozygous PLOD2 mutations

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Introduction
Bruck syndrome (BS) is a congenital disorder characterized by joint flexion contractures, skeletal dysplasia and increased bone fragility, showing strong clinical overlap with Osteogenesis Imperfecta (OI). On a genetic level, BS is caused by bi-allelic mutations in either the *FKBP10* or the *PLOD2* gene. *PLOD2* encodes the lysyl hydroxylase 2 (LH2) enzyme which is responsible for the hydroxylation of lysyl residues in fibrillar collagen telopeptides. This modification is essential for allowing collagens to form stable intermolecular cross-links in the extracellular-matrix. Bone collagen has a unique pattern of cross-linking that is required for bone strength, resistance to microdamage and crack propagation and also a normal ordered pattern of mineral nanocrystals in the collagen fibrils. To date, no direct studies of human bone from BS caused by *PLOD2* mutations have been reported. We present here results from a case of BS in a 4-year-old Caucasian patient, caused by compound heterozygous mutations in *PLOD2*.

Materials and Methods
Bone tissue was collected during surgery, and bone collagen was biochemically characterized using SDS-PAGE, cross-link analysis and peptide mass-spectrometry.

Results
The patient's bone showed diminished hydroxylation of type I collagen telopeptide lysines, while hydroxylation at helical sites was unaltered. Consequently, mature trivalent cross-links, which depend on the presence of telopeptide hydroxylysines, were shown to be greatly reduced. Mass-spectrometry identified abundant allysine aldol dimeric cross-links in the patient's bone, which are not normally present in bone but are a feature skin collagen. SDS-PAGE further illustrated a skin-like migration pattern of the patient's extracted bone collagen, with more prevalent β-dimers and γ-trimers. Type II collagen cross-linked peptides from the patient's urine were also analyzed. In contrast to bone type I collagen, the results showed a normal telopeptide lysine hydroxylation of cartilage type II collagen.

Discussion
Taken together these findings shed light on the complex mechanisms that control the unique posttranslational chemistry and cross-linking of bone collagen, and that when defective can cause a brittle bone disorder.

P144, NOX inhibition reverses the persistent fibrotic phenotype of lesional scleroderma fibrolasts

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Introduction
Reactive oxygen species (ROS)/nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) may contribute to fibrogenic responses. Here, we examine if the antioxidant N-acetylcysteine (NAC), the NOX inhibitor diphenyleneiodonium (DPI) and the selective NOX1/NOX4 inhibitor GKT-137831 impairs profibrotic gene expression in human dermal (HDF) fibroblasts. We also assess if GKT-137831 can block the persistent fibrotic phenotype of lesional scleroderma (SSc) fibroblasts from patients with early onset diffuse disease.

Materials and Methods
.We use real-time polymerase chain reaction and Western blot analysis to evaluate whether NAC and DPI impair the ability of TGFbeta1 to induce expression of fibrogenic genes in fibroblasts. The effects of GKT-137831 on TGFbeta-induced protein expression and the persistent fibrotic phenotype of lesional scleroderma (SSc) fibroblasts were tested using Western blot and collagen gel contraction analyses.

Results
TGFbeta1 induces CCN2, CCN1, endothelin-1 and alpha-smooth muscle actin (SMA) in a fashion sensitive to NAC and DPI. GKT-137831 impaired TGFbeta-induced CCN2 and alpha-SMA protein expression in HGF and HDF. In lesional SSc dermal fibroblasts, GKT-137831 reduced alpha-SMA and CCN2 protein overexpression and collagen gel contraction

Discussion
These results are consistent with the hypothesis that antioxidants or NOX1/4 inhibition may be useful in treating SSc.

P145, Missense and Nonsense mutations in Collagen IV elicit changes in endothelial cell mediated vascular function

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Introduction
Missense mutations in *COL4A1* and *COL4A2* cause familial forms of eye, kidney and cerebrovascular disease including small vessel disease, intracerebral haemorrhage and porencephaly. These mutations are thought to act by causing matrix defects and endoplasmic reticulum stress. Several nonsense mutations, predicted to affect protein levels have also been identified and common variants in COL4A1/2, also likely to affect collagen gene expression, are associated with arterial stiffness, coronary artery disease and sporadic intracerebral haemorrhage in the general population. However, the mechanism of these common variants and nonsense mutations remains enigmatic.

Materials and Methods
We employed a novel heterozygous Col4a2 knockout mouse model to determine the pathogenicity of reduced Col4a2 expression. Histopathological examination determined morphological defects and intracerebral haemorrhaging. Vascular structure/function was determined using wire and pressure myography, and compared to mice with a *Col4a1* missense mutation ($Col4a1^{+/svc}$).

Results
$Col4a2^{+/-}$ animals have a deletion in exon 18 leading to a premature stop codon, causing a 50% reduction in mRNA levels. Histopathological analysis revealed no gross defects affecting the eye and brain in 3 month old $Col4a2^{+/-}$ mice. Surprisingly, no signs of intracerebral haemorrhaging was detected. Analysis of vascular function revealed normal constriction to noradrenaline, in contrast to $Col4a1^{+/SVC}$. However, both models displayed increased endothelium-dependent nitric oxide (NO) mediated relaxation. Treatment of vessels with inhibitors against NO synthase and Calcium-dependent potassium channel (Kca) identified an increased reactivity of Kca channel mediated relaxation in $Col4a1^{+/svc}$ and nitric oxide synthase dysfunction in $Col4a2^{+/-}$. In addition, no difference was observed in vascular structure of $Col4a2^{+/-}$ versus wildtype littermates. $Col4a1^{+/svc}$ develop hypertophy with outward remodelling.

Discussion
Phenotypic analysis revealed that reduced COL4A2 expression levels result in a much milder phenotype compared to missense mutations. Our data also provide evidence for an important role of the basement membrane in maintaining vascular and endothelial cell function with mutation specific effects**.** Therefore $Col4a2^{+/-}$ may represent a powerful animal model to delineate milder more common, probably adult onset diseases associated with collagen IV.

P146, Modelling Alport syndrome in zebrafish

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Introduction
Mutations in human *COL4A3*, *COL4A4* and *COL4A5* genes lead to improper assembly of the α3α4α5(IV) collagen network in basement membranes. Such mutations lead to Alport syndrome, where the composition and function of basement membranes that utilise the α3α4α5(IV) collagen network is compromised. The renal blood filter (glomerulus) is enriched with α3α4α5(IV) collagen, which is deposited by specialised epithelial cells called podocytes to generate the glomerular basement membrane (GBM). As a consequence, patients with Alport syndrome initially present with glomerular dysfunction, manifesting as haematuria and proteinuria in early childhood. Current treatment is with angiotensin converting enzyme (ACE) inhibitors to delay the progression of chronic kidney disease (CKD) and the requirement for renal dialysis or transplantation. Our understanding of the mechanisms of CKD progression in Alport syndrome is limited and new models are required to provide further insight into mechanisms of disease with the aim of generating novel treatments. Therefore we are developing a zebrafish model of Alport syndrome in order to exploit this simple and tractable system to enable a better understanding of Alport syndrome and cell-matrix biology in the glomerulus.

Materials and Methods
We analysed gene expression for *col4a3*, *col4a4* and *col4a5* by *in-situ* hybridisation in zebrafish and performed immunohistochemical analysis using a pan collagen type IV antibody on the zebrafish GBM. We also used CRISPR-Cas9 knockout fish for *col4a3*, *col4a4* and *col4a5* to develop models of the human disease.

Results
We find *col4a3*, *col4a4* and *col4a5* are expressed in the zebrafish glomerulus and antibody staining demonstrated collagen IV proteins are present within the zebrafish GBM. Knockout fish have been bred to homozygosity and are viable. Ongoing analyses will determine what effects these knockouts have on glomerular function and if they correlate with the phenotypes observed in the human disease.

Discussion
Taken together, our data show that the α3α4α5(IV) collagen network is present in the zebrafish GBM and suggests that zebrafish can be used as an *in vivo* model of Alport syndrome.

P147, Live optical super-resolution microscopy applied to the characterization of maturation, localization and trafficking of defective dystroglycan mutants

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Introduction
The ubiquitous dystroglycan (DG) adhesion complex is located at the plasma membrane where it provides an essential link between the extracellular matrix and the cytoskeleton. Dystroglycan is expressed as a pro-peptide which undergoes a series of maturation steps whereby the precursor is cleaved into an a (extracellular) and b (transmembrane) subunit and heavily glycosylated. Dysfunctional dystroglycan complexes are linked to several autosomal recessive neuromuscular disorders such as severe congenital (Muscle-Eye-Brain and Walker-Warburg syndrome) or limb-girdle muscular dystrophies (LGMD2P). Moreover, genetic abnormalities of glycosyltransferases leading to hypoglycosylated a-DG result in a reduced affinity towards the extracellular matrix protein laminin-2. Primary dystroglycanopathies are those caused by direct missense mutations of the dystroglycan core protein, and we are establishing models for studying them with a multidisciplinary approach.

Materials and Methods
We use a combination of several fluorescence microscopy techniques, from advanced to super-resolution fluorescence microscopy, such as 3D structured illumination. For that purpose, the dystroglycan subunits are individually fluorescently labeled. Wild-type DG expression is compared to site-directed missense mutations in live cells.

Results
Live cell super-resolution microscopy allows us to efficiently and reliably track DG's subcellular localization. Trafficking of the dystroglycan complex towards the plasma membrane during processing, as well as some retrograde movements, are observed.

Discussion
This detailed imaging approach illustrates the severe molecular and trafficking defects in dystroglycan mutations involved in LGMD2P and muscle-eye-brain disease. We believe that our experimental work can be crucial to understand the complex maturation pathway of dystroglycan and its subunits within the cell.

P148, Structural model of domain 1 of the a-dystroglycan glycosylating enzyme LARGE

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Introduction
Dystroglycanopathies are characterised by hypoglycosylation of a-dystroglycan (DG) that affects binding to extracellular matrix molecules both in muscle and brain. Dystroglycanopathies depending on an aberrant O-glycosylation of a-DG are associated to several genes, including POMT1/2, FKTN, FKRP, POMGNT2 and LARGE. LARGE is a two-domains glycosyltransferase whose function is to add multiple repetitions of a disaccharide responsible for laminin binding. The structural features of LARGE and how it interacts with a-DG remain still unknown. Here, we present the first structural model of domain 1 of LARGE that we also used as a structural basis to evaluate the effects of a missense mutation (S311F) resulting in congenital muscular dystrophy with brain and eye anomalies.

Materials and Methods
Two fold-recognition algorithms (I-TASSER and HHPRED) have been employed to build the three-dimensional structure of LARGE domain 1. The Build Mutant protocol implemented in Discovery Studio (Biovia) was used to generate a structural model of the S331F mutant. The stability of the wild-type protein and the effects of the mutation have been further investigated by means of molecular dynamics (MD) simulations using the program Desmond.

Results
The first-ranking structures from the two fold-recognition algorithms indicate the same fold, corresponding to the one of galactosyltransferase LgtC. The S311F replacement results in an increase of hydrophobicity in the vicinity of the catalytic site, and MD simulations clearly point out an altered flexibility of the backbone of the enzyme upon mutation. The model has also been used to set up a preliminary recombinant expression system of domain 1.

Discussion
We employed molecular modelling studies to generate a reliable model structure of the domain 1 of LARGE. The model represents the structural basis for computational mutagenesis studies, for model building of the entire protein and for protein-protein docking with α-DG.

P149, Implications of Gas1 in Synovial Joint Formation and Disease Mechanism of Brachydactyly Type A1

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Introduction
Brachydactyly type A1 (BDA1) is a congenital disease causing reduced digit lengths and a missing joint in digit V, resulting from homozygous E95K mutation in Ihh gene. IhhE95K has expanded signaling field, thus higher Ihh concentration in developing joint. Preliminary studies show initiation but incomplete development of joint primordia leading to a missing joint, concomitant with decrease in cell death. Growth arrest specific-1 (Gas1) is a joint expressing HH binding protein shown to have dependency receptor characteristics in the absence of HH inducing apoptosis. Gas1 is expressed in the developing joint. We hypothesize that increased level of IHH in BDA1 joints reducing cell death as a development mechanism for the missing joint.

Materials and Methods
Joint development in BDA1 mouse embryos were systematically analysed for differentiation and cell death markers in relation to Gas1. *Ex vivo* joint cultures are subjected to treatment with general caspase inhibitor zFAD-FMK, to assess the significance of apoptosis in joint formation. To understand the role of Gas, a conditional mouse for an activation of Gas1 was generated.

Results
We confirmed all phalangeal interzones are initiated in BDA1 mice. However, some distal interzones in BDA1 mice failed to progress and a joint is not formed, causing brachydactyly. TUNEL signals are detected in the centre of Gas1 expressing digit interzones associated with the cavitation event, correlating to development progression of the individual joints. Cleaved caspase3 is detected but much less in comparison to TUNEL. However, *ex vivo* joint cultures treated with zFAD-FMK, an apoptosis inhibitor did not prevent joint cavitation in wt mice, suggesting alternative modes of cell death, such as necroptosis. In BDA1, despite Gas1 expression remains in interzone, TUNEL is reduced that is not significant in distal joints, consistent with the phenotype.

Discussion
A relationship between cell death, joint cavitation and BDA1 phenotype was demonstrated. Whether cell death is associated with canonical apoptotic pathways needs to be further studied. The potential of Gas1 in regulating the cell death event and cavitation in joint formation will be tested as we have generated a conditional mouse for an activation of Gas1 and the level of Ihh in disease mechanism of BDA1.

P150, Extracellular matrix disarray and αvβ3 integrin-ILK-Snail1-mediated signaling are involved in a fibroblast-to-myofibroblast transition in hypermobile Ehlers-Danlos syndrome

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Introduction
During tissue injury, fibroblasts differentiate to myofibroblasts regulating synthesis, remodeling, reabsorption of the extracellular matrix (ECM), and inflammation's resolution. The organization of the α-smooth muscle actin (α-SMA) cytoskeleton allows myofibroblasts' migration and ECM contraction. The persistent myofibroblasts' activity contributes to chronic inflammation.
Ehlers-Danlos syndromes (EDS) are a heterogeneous group of heritable connective tissue disorders sharing skin hyperextensibility, joint hypermobility (JHM), and tissue fragility. Classical EDS (cEDS), caused by defects in type V collagen (COLLV), is characterized by marked cutaneous involvement and generalized JHM. Vascular EDS (vEDS), due to COLLIII defects, is characterized by arterial rupture/dissection/aneurysm and organ ruptures. Hypermobile EDS (hEDS), orphan of a genetic etiology, is primarily identified as having generalized JHM, related musculoskeletal manifestations, and a mild skin involvement. The hEDS-associated phenotypic spectrum is broad and includes multiple signs and symptoms shared with chronic inflammatory systemic diseases. To shed light into pathomechanisms underlying hEDS, we performed gene expression profiling of hEDS dermal fibroblasts together with a detailed cellular characterization of these cells in comparison with those of cEDS and vEDS.

Materials and Methods
Transcriptome-wide expression profiling was carried out by microarray technology. ECM and integrins organization, α-SMA-cytoskeleton, inflammatory markers, and the integrin-mediated transduction pathway were investigated by immunofluorescence microscopy and Western blotting. The migratory capability was measured by the Transwell migration assay.

Results
The hEDS cells showed significant expression changes of several genes involved in maintenance of ECM organization/homeostasis and in immune, inflammatory, and pain responses. These cells shared with cEDS and vEDS fibroblasts the disassembly of COLLs-, fibrillins-, fibronectin (FN)-ECM and the organization of the αvβ3 integrin. Only in hEDS cells, the ECM disarray was associated with increased levels of the metalloproteinase-9, presence of proteolytic FN-fragments in cells' media, organization of the α-SMA-cytoskeleton, enhanced migration, altered expression of the inflammation mediators CCN1 and CTGF, and an ILK-mediated αvβ3 integrin signaling involving the transcription factor Snail1, consistent with a fibroblast-to-myofibroblast transition.

Discussion
The myofibroblast-like phenotype distinguishes hEDS from cEDS and vEDS fibroblasts and suggests an *in vitro* inflammatory-like condition, which correlates well with the systemic clinical manifestations of the patients.

P151, Prevention of Vascular Calcification by Growth Hormone-Releasing Hormone Agonists

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Introduction
Vascular calcification (VC) is a marker of the severity of atherosclerotic disease. Hormones play important roles in regulating calcification; estrogen and parathyroid hormones exert opposing effects, the former alleviating VC and the latter exacerbating it. So far no treatment strategies have been developed to regulate clinical VC. Growth hormone-releasing hormone (GHRH) is a neuropeptide produced by the hypothalamic neurons as well as other peripheral cells. The effects of GHRH and its agonist (GHRH-A) on the blocking VC were investigated in cultured smooth muscle cells (SMCs) and a mouse model.

Methods and Results
Young adult osteoprotegerin deficient ($OPG^{-/-}$) mice were given daily subcutaneous injections of GHRH-A (MR409) for 4 weeks. Calcification of the aorta was significantly reduced in the treated mice, which was paralleled by significantly lower alkaline phosphatase (ALP) activity and a dramatic reduction in the expression of transcription factors, the osteogenic marker gene Runx2 and its downstream factors, such as osteonectin and osteocalcin. The mechanism of action of GHRH-A was dissected in SMCs isolated from human and mouse aortas. SMC calcification induced by osteogenic medium was inhibited in the presence of GHRH or MR409, as evidenced by reduced ALP activity and Runx2 expression. Inhibition of calcification by MR409 was partially reversed by MIA602, a GHRH antagonist, or a GHRH receptor selective siRNA. Treatment with MR409 invoked elevated cytosolic cAMP and its target, protein kinase A (PKA) which in turn blocked NADPH oxidase activity and reduced reactive oxygen species (ROS) production, thus blocking the phosphorylation of NFκB (p65), a key intermediate in the RANKL-Runx2/ALP osteogenesis program. These beneficial effects of MR409 were abolished by a PKA-selective siRNA or the chemical inhibitor H89.

Discussion
GHRH-A controls osteogenesis in SMCs through cross talk between PKA and NFkB (p65) by the suppression of ROS production that induces the Runx2 gene and ALP, thereby blocking inflammation-mediated osteogenesis. GHRH-A may represent a new pharmacological strategy to regulate VC.

Workshop 7: The Immunology/Matrix Interface

P152, Investigating the tumour ECM in the inhibition of anti-tumour immunity

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Introduction
Extracellular matrix (ECM) degradation and remodelling occurs in invasive malignant progression, resulting in the expression of unusual matrix proteins in the tumour microenvironment (TME). Whether the remodelled ECM hinders or promotes tumour progression remains controversial. In our work, we identified a tumour matrix index (MI) that correlates with an immunosuppressive phenotype that is conductive of tumour progression. In this work we aim to investigate the direct effect of tumour ECM on immune cell phenotype.

Materials and Methods
HGSOC patient tissue is decellularized to generate an *in vitro* 3-D tissue platform for immune cell co-cultures using human macrophages, later work will focus on other immune cells. Decellularized tissue is characterised by matrisome proteomics, IHC, H&E and assigned a disease score using Definiens® digital image analysis. Flow cytometry using a panel of macrophage antibody markers, qPCR and IHC is used to characterize macrophage phenotype. Proteomics informed by transcriptomics (PIT) is used to identify tumour specific MI protein isoforms expressed in decellularized tissues.

Results
Decellularized tissues maintain ECM integrity and the MI. Monocytes or macrophages can be incubated with decellularized tissues, and later isolated from the tissue for flow or qPCR analysis. Direct macrophage-decellularized tissue interaction can be assessed by IHC and IF.

Discussion
Decellularized human tissue provides a platform to study immune cell – ECM interactions. From our data so far, matured macrophages are viable for over 9 days and can be phenotyped after co-culture end points. We are currently determining how diseased tissue influences macrophage phenotype. This work may also be useful for other diseases associated with fibrosis.

P153, Human Rhinovirus Infection of Airway Epithelial Cells Induces Tenascin-C Release

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Introduction
Viral infections are the cause of 75% of all asthma exacerbations, with human rhinovirus (HRV) being the most common. Tenascin-C (TNC) is an ECM protein that is present in small quantities in the airway of healthy individuals, but in high quantities in asthma sufferers, however, the inflammatory potential of TNC in asthma has yet to be investigated. We hypothesise that HRV infection induces the upregulation of TNC expression in the airway, contributing to increased inflammatory cytokine production.

Materials and Methods
C57BL/6 mice were intranasally administered with the viral mimic Poly(I:C) and bronchoalveolar lavage fluid (BALF) analysed for TN-C. BEAS-2B cell line and primary human bronchial epithelial cells (PBECs) were stimulated with Poly(I:C) or infected with HRV and assayed for TN-C mRNA, protein expression and release. Finally, recombinant TN-C was added exogenously to BEAS-2B cells and extracellular vesicles (EVs) were isolated following stimulation, analysed for TN-C expression and used to stimulate BEAS-2B cells and macrophages.

Results
TNC expression in the mice BALF was significantly upregulated following Poly(I:C) stimulation, and *in vitro* Poly(I:C) and HRV treatment induced TN-C mRNA and TN-C release in PBECs and BEAS-2B cells, highlighting a novel relationship between HRV infection and airway TN-C expression. Viral-induced TN-C release was significantly higher from asthmatic PBECs compared to non-asthmatic PBECs, demonstrating an increased prominence in a disease setting. Furthermore, Poly(I:C) stimulation also induced EV release and EV-associated TNC expression in BEAS-2B cells. EVs derived from Poly(I:C) stimulation induced cytokine release from BEAS-2B cells and macrophages, as did stimulation with recombinant TN-C, demonstrating the inflammatory potential of EVs and TN-C in the airway following viral treatment.

Discussion
Further work to determine the effects of TNC on inflammation in the airway is ongoing. The current data support a potential link between TNC release following HRV infection and inflammatory cytokine production in the airway, potentially through the release of EVs.

P154, ST: TSG-6 modulates chondrocyte phenotype in osteoarthritis by supressing inflammatory signals that promote cartilage matrix breakdown

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Introduction

TSG-6 is a secreted protein expressed during inflammation that has diverse tissue-protective and anti-inflammatory activities. TSG-6's ability to modulate the interactions of matrix molecules with cell surface receptors (e.g. HA/CD44 binding) and with immune regulators (e.g. HSPG/chemokine binding) likely underpins many of its beneficial properties. Here we investigated the role of TSG-6 as an inhibitor of cartilage damage in the context of osteoarthritis (OA).

Materials and Methods

Human chondrocytes, derived from bone marrow stem cells, were cultured as 3D pellets in the presence of IL-1b or TNF ± recombinant human (rh)TSG-6 or its isolated Link module domain (Link_TSG6); ADAMTS5 and MMP13 gene expression were determined by quantitative PCR. Cartilage explants from OA patients undergoing knee arthroplasty were cultured in the absence/presence of IL-1b/oncostatin-M ± rhTSG-6 or Link_TSG6; glycosaminoglycan loss was quantified. RNAscope probes were used to quantitate expression of TSG-6, ADAMTS5 and MMP13 mRNAs by chondrocytes in OA cartilage.

Results

Link_TSG6 and rhTSG-6 supressed cytokine-induced gene expression of the proteases MMP13 and ADAMTS5 (key mediators of cartilage breakdown in OA) by cultured chondrocytes, where Link_TSG6 was substantially more potent than the full-length protein. Consistent with this, the loss of proteoglycans from human OA cartilage explants was significantly inhibited by Link_TSG6 treatment. RNAscope experiments revealed a negative correlation between TSG-6 and MMP13 expression by chondrocytes in the superficial regions of damaged OA cartilage; however, we saw a positive correlation between the expression of TSG-6 and ADAMTS5.

Discussion

Our data reveal that TSG-6 can modulate chondrocyte phenotype by suppressing cellular responses to inflammatory signals, thereby reducing OA-associated cartilage damage. We are now investigating potential mechanisms for TSG-6's inhibition of MMP13 expression and for its effects on ADAMTS5 activity, which might involve regulation of both cytokine-induced expression and receptor-mediated endocytosis. TSG-6 has been shown in other systems to mediate anti-inflammatory effects in a CD44-dependent manner, such that TSG-6's enhancement of CD44/HA interactions might represent a mechanism for its regulation of chondrocyte phenotype. Alongside this, we are developing Link_TSG6 as a biological to treat OA by harnessing the intrinsic protective properties of TSG-6 in cartilage.

P155, ST: Leukocyte migration and inflammatory disease: the collaboration between endothelial sugars and chemokines

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Introduction
Chemoattractant cytokines (chemokines) are integral in recruitment of leukocytes to inflammatory sites and associated inflammatory pathologies, e.g. autoimmune diseases. Chemokine function is dependent on their ability to bind to, and be presented on, cell surface glycoasminoglycans (GAGs), mediating the formation of chemotactic gradients, enabling presentation and signaling through receptors on leukocytes. GAGs are integral extracellular matrix components that not only support chemokine localisation but also help to form the glycocalyx, which acts as a physical barrier to leukocyte adhesion and subsequent migration. Chemokine oligomerization is essential to *in vivo* chemokine mediated leukocyte recruitment (e.g. CCL2 and CCL5), however, monomeric chemokine is largely sufficient for receptor signaling, leaving the mechanistic importance of oligomerization unresolved.

Materials and Methods
We have utilized surface plasmon resonance (SPR) and quartz crystal microbalance with dissipation (QCMD) alongside endothelial binding to examine the interaction of chemokines, and mutants, with different isolated GAGs and the cell surface.

Results
These results demonstrate that the majority of chemokines bind to heparin and heparan sulfate (HS) with a range of affinities, and more selectively with chondroitin sulfate-A. Inhibition of chemokine oligomerization suggests that this process mediates the nature, strength and selectivity of some chemokine-GAG interactions. Our data also suggests that chemokines have different abilities to cross-link individual HS chains on a biosensor. Importantly, this process may provide a novel mechanism that enables retention of chemokines on the cell surface and subsequent modification of the glycocalyx. Chemokine mediated HS cross-linking also appears to be dependent upon oligomerization, in the case of CCL2, CCL5 and CXCL4. Therefore, there appears to be an overlap between GAG-binding, HS chain cross-linking and chemokine oligomerization

Discussion
We conclude that oligomerization enables binding to, and modification of, GAG chains, enhancing retention on cell surfaces whilst also enabling chemokine mediated GAG re-organization. This enables chemokine cell-surface localization under flow and thus leukocyte recruitment *in vivo* and potentially suggests a novel function of chemokines in physical re-organization of the glycocalyx.

P156, ST: Leukocyte control of matrix deposition in the lymph node

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Introduction
To orchestrate the adaptive immune response, lymph nodes (LNs) require a highly-organised tissue architecture supported by stromal cell populations. Fibroblastic reticular cells (FRCs) comprise the most abundant LN stromal cells. FRCs express podoplanin (PDPN), only known endogenous ligand of the C-type lectin receptor CLEC-2. These specialised fibroblasts produce and enwrap the conduit, a tubular network of collagens and associated proteins that controls intranodal flow of lymph-borne factors. LNs expand 3-5 times in size with each immune response, but whether these structures are remodelled during the rapid and reversible expansion of LNs is unknown. During LN expansion, migratory dendritic cells (DCs) expressing CLEC-2 inhibit contractility the FRC network, allowing initial expansion of the LN. Here, we study that this same interaction also regulates remodelling of the extracellular matrix of LNs.

Materials and Methods
Conduit network integrity was studied ex vivo by different histology techniques. Phosphoproteomic and transcriptomic analysis was used to study how CLEC-2 binding to PDPN+ FRCs regulates signalling cascades controlling matrix deposition and expression of matrix components. Functional *in vitro* assays and 3D DC-FRC co-cultures were used to demonstrate mechanisms involved.

Results
Histological analysis shows a marked reduction in collagens within the conduit network in LNs during the immune response. By RNAseq we found that FRCs express >400 matrisome genes, of which nearly 40% were regulated by CLEC-2/PDPN interactions, including downregulation of Col6a1, Col6a2, Col4a5 and Col4a6. We characterised *in vitro* FRC-derived 3D matrices by immunofluorescence and mass spectrometry, finding that CLEC-2 reduces total matrix deposition and modifies fibre bundling and alignment. Mechanistically, we found that CLEC-2 expressed by DCs disrupts focal adhesion in FRCs affecting microtubule attachment to the membrane, essential for localized matrix deposition.

Discussion
Our data demonstrate a novel mechanism by which DCs regulate expression and deposition of extracellular components by fibroblasts. This mechanism seems to act regulating the conduit network in draining LNs after immunization. Loss of fibrillar components may reduce organ stiffness and therefore facilitate rapid LN expansion. It is therefore likely that, function of the conduit network will be compromised during the onset of the immune response.

P157, Spontaneous atopic dermatitis due to immune dysregulation in mice lacking Adamts2 and 14

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Introduction
Since its first description, ADAMTS14 has been considered as an aminoprocollagen peptidase based on its high similarity with ADAMTS3 and ADAMTS2.

Materials and Methods
Since the importance of ADAMTS14 for procollagen processing was never experimentally demonstrated *in vivo*, we generated *Adamts14*-deficient mice.

Results
These mice are healthy, fertile and display normal aminoprocollagen processing. They were further crossed with *Adamts2*-deficient mice to evaluate potential functional redundancies between these two highly related enzymes. Initial characterizations made on young *Adamts2-Adamts14*-deficient animals showed the same phenotype as that of *Adamts2*-deficient mice, with no further reduction of procollagen processing and no significant aggravation of the structural alterations of collagen fibrils. However, when evaluated at older age, *Adamts2-Adamts14*-deficient mice surprisingly displayed epidermal lesions, appearing in 2 month-old males and later in some females. Immunohistological evaluations of skin sections around the lesions revealed thickening of the epidermis, hypercellularity in the dermis and extensive infiltration by immune cells. Additional investigations, performed on young mice before the formation of the initial lesions, revealed that the primary cause of the phenotype was not related to alterations of the epidermal barrier but was rather the result of an abnormal activation and differentiation of T lymphocytes towards a Th1 profile. However, the primary molecular defect probably does not reside in the immune system itself since irradiated *Adamts2-Adamts14*-deficient mice grafted with WT immune cells still developed lesions.

Discussion
While originally created to better characterize the common and specific functions of ADAMTS2 and ADAMTS14 in extracellular matrix and connective tissues homeostasis, the *Adamts2-Adamts14*-deficient mice revealed an unexpected but significant role of ADAMTS in the regulation of immune system, possibly through a cross-talk involving mesenchymal cells and the TGFβ pathways.

P158, ST: Post-translational modifications of the extracellular matrix: key events in disease pathogenesis

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The extracellular matrix (ECM) is both complex and dynamic; its composition and structure vary from one tissue to the next, and it is profoundly altered throughout development, as we age, and during disease. One key factor defining ECM content is post-translational modification of its protein constituents. However, surprisingly little is known about how the matrix is modified, and how this impacts ECM function. Citrullination, the conversion of the amino acid arginine to citrulline, is significantly elevated at sites of inflammation. My work investigates how this post-translational modification of ECM proteins shapes innate and adaptive immune responses, and how these processes contribute to pathological autoimmunity in rheumatoid arthritis (RA).
Distinct citrullinated sites on the matrix molecule tenascin-C were defined by mass spectrometry, and the functional consequences of these modifications on immune cell behaviour investigated. Citrullinated tenascin-C provoked elevated innate immune responses, creating a mobile, aggressive macrophage phenotype, by reducing cell adhesion and enhancing release of a unique signature of pro-inflammatory cytokines. Peptide mapping and protein mutagenesis identified the epitope within tenascin-C responsible for these effects. Targeted inhibition of inflammatory signalling pathways revealed a complex interplay between the immune sensor toll-like receptor 4 and macrophage integrins that is essential for the resolution of inflammation, but which citrullinated tenascin-C escapes, due to the loss of key arginines in integrin binding sites.
I also defined different citrullinated sites on tenascin-C that activate adaptive immunity in people with RA. These modified epitopes were capable of breaking immune tolerance, generating citrulline- specific pathogenic autoantibodies that are the hallmark of this disease. Autoantibody responses towards one immunodominant epitope were examined in large patient cohorts and found to be an accurate means by which to diagnose established RA, as well as identifying people at risk of developing disease, years before the detection of clinical symptoms.
My data demonstrate that ECM post-translational modification drives aberrant inflammation via multiple mechanisms; through breaking immune tolerance and the formation of pathogenic autoantibodies, and by activating innate pathways in manner that is no longer subject to homeostatic control. Understanding more about matrix modification therefore can provide novel approaches to disease diagnosis and treatment.

P159, CXCL6: a glycosaminoglycan-binding chemokine with pro-inflammatory activities that are modulated by heparin and TSG-6

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Introduction
CXCL6 plays a role in cartilage homeostasis, where this chemokine may be retained in the chondrocyte pericellular matrix via its interaction with heparan sulphate proteoglycans (HSPGs). In osteoarthritis (OA), the release of CXCL6 from cartilage matrix is thought to reduce chondrocyte viability and has been hypothesised to attract neutrophils, leading to further tissue damage. Here we compared the interactions of CXCL6 and CXCL8 with glycosaminoglycans and their effects on leukocyte migration. We also investigated whether TSG-6 (a tissue-protective protein made during inflammation) interacts with CXCL6 and how this modulates its function.

Materials and Methods
Plate-based assays and affinity chromatography were used to characterise chemokine-glycosaminoglycan interactions. Chemotaxis and transendothelial migration experiments were done using Transwell assays. WT and mutant Link_TSG6 proteins were expressed in *E. coli* and their interactions with CXCL6 (and other chemokines) were analysed by Surface Plasmon Resonance.

Results
CXCL6 (like CXCL8) interacted with heparin and highly sulphated HS but exhibited essentially no binding to other glycosaminoglycans. In Transwell assays, CXCL6 promoted chemotaxis and transendothelial migration of HL-60 cells (a neutrophil cell-line) and of a pre-B cell-line expressing the CXCR2 receptor. Additionally, soluble heparin/HS inhibited CXCL6 chemotaxis activity while the effect on CXCL8 was minimal. The Link module of human TSG-6 (Link_TSG6) was found to bind to CXCL6, where this interaction inhibited the association of CXCL6 with heparin (as is the case for some other chemokines) but didn't interfere with binding to the CXCR2 receptor. As a result, Link_TSG6 reduced CXCL6-mediated transendothelial migration but not chemotaxis. Additionally, a mutant of Link_TSG6 was identified that had impaired binding to CXCL8 and also abolished Link_TSG6's inhibitory effect on CXCL8-mediated transmigration. This Link_TSG6 mutant also had reduced binding affinity for CCL2 and CXCL6 (known ligand of TSG-6).

Discussion
Here we have shown that the promotion of chemotaxis by CXCL6 (unlike CXCL8) is inhibited by heparin/HS. These data suggest that degraded HS chains would inhibit CXCL6's pro-inflammatory effect, e.g. in OA cartilage. We found that Link_TSG6 (which we are developing as a biologic for OA) also inhibits CXCL6-mediated leukocyte migration and that there may be a common chemokine binding site on TSG-6.

P160, Role of Interleukin-13 in the Extracellular Matrix Turnover during *Nippostrongylus brasiliensis* Infection

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Introduction
Fibrosis is an excessive and disorderly accumulation of insoluble collagen fibres as the result of tissue injury, causing a loss of tissue specialised function. Infection with the rodent nematode, *Nippostrongylus brasiliensis* is an established model to evaluate repair of acute lung injury caused by nematode migration through the parenchyma. Lung repair is dependent on the T helper 2 (Th2) cell cytokines interleukin -4 (IL-4) and -13 (IL-13), which interact with the shared IL-4Ra receptor subunit. It has been previously demonstrated that during skin repair, IL-4Ra signalling to macrophages induces expression of Resistin-like molecule alpha (Relm-a), which in turn induces the expression of the collagen cross-linking enzyme lysyl hydroxylase 2 (LH2). The specific role of IL-13 in lung repair has not been investigated yet during *N. brasiliensis* infection.

Materials and Methods
C57BL/6 wild-type and *Il13-/-* mice were infected with 250 infective larvae of *N. brasiliensis*. Lungs and bronchioalveolar lavages (BAL) were harvested 2, 4, and 6 days after infection. Gene expression profile, flow cytometry, immunohistochemistry and ELISA were performed.

Results
IL-13 regulated infiltration of neutrophils and eosinophils in *N. brasiliensis* infection. Relm-a expression in lungs and abundance in BAL was increased upon *N. brasiliensis* infection and was controlled by IL-13. Additionally, the expression of the transmembrane matrix metalloproteinase MMP-14 was regulated by IL-13 in lungs.

Discussion
These data suggested that, during injury, IL-13 might regulate the extracellular matrix turnover via Relm-a. This study gives important insights to fully understand IL-13's pro-fibrotic activities and the link between type 2 immunity and initiation of pro-fibrotic pathways.

P161, Up-Scale Manufacture of Chondrocytes in the Development of Allogeneic Cartilage Therapies

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Introduction

Autologous chondrocyte implantation (ACI) is the first UK manufactured cell therapy to be approved by the National Institute for Health and Care Excellence. However, there is a requirement for this procedure to become more cost-efficient if it is to reach a wider patient population. An allogeneic product would help to improve the cost effectiveness and wide-spread use of chondrocyte therapy.

Materials and Methods

Macroscopically normal cartilage from three donors undergoing knee arthroplasty was used to derived chondrocyte cultures in media supplemented with human platelet lysate (HPL, Stemulate®) or foetal bovine serum (FBS) on tissue culture plastic (TCP) or the Quantum® Cell Expansion System (Terumo BCT) (HPL only). The Quantum® is a computerised incubator which houses disposable bioreactors consisting of ~11.5k fibres, providing a growth surface of ~2.1m^2. Total cell yield and the number of population doublings were calculated. Flow cytometry analysis was used to assess mesenchymal stromal cell, integrin and chondrogenic potency immunoprofiles on freshly isolated, Quantum® and TCP cultures. Chondrogenesis was assessed using standard pellet culture and glycosaminoglycan (GAG) assays.

Results

Chondrocytes grown in FBS cf. HPL had distinct rounded/cuboidal compared to biopolar/fibroblastic morphologies. The Quantum® produced higher cell yields (75±38 x10^6 cells) cf. FBS supplemented (2.6±0.4 x10^6 cells) or HPL supplemented (6.9±38.9 x10^6 cells) TCP cultures. The number of population doublings for Quantum®, TCP FBS and TCP HPL being 2.82±1.18, 1.57±1.1 and 1.3±0.12, respectively. Quantum® chondrocytes displayed similar surface immunoprofiles to those grown on TCP and unlike freshly isolated cells, were immunopositive for CD90, CD73, CD105, CD166, CD151 and all integrins tested. Chondrogenic pellet analysis demonstrated that all cultures produced GAGs to varying degrees.

Discussion

This study was conducted to test the Quantum® for rapid chondrocyte culture expansion. Our preliminary results show that compared to traditional methods, the bioreactor is capable of generating high numbers of chondrocytes, which demonstrate comparable characteristic immunoprofiles and cartilage forming capacity. The Quantum® has the potential to reduce manufacturing costs, decrease the risk of product contamination and improve reproducibility for multiple dose chondrocyte banking. Further work will examine the effects of Quantum® expansion on gene expression profiles, telomere length and evaluate the cartilage forming capacity of cells in preclinical models of cartilage injury.

P161(2) **Developing physiological, 3D extracellular matrices to study macrophage gene regulation**

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Introduction

MicroRNAs (miRNAs) are small non-coding RNAs that bind to complementary mRNA sequences to post-transcriptionally regulate gene expression during development, homeostasis and disease. miRNAs also control mammalian immunity from hematopoietic lineage development to immune responses to pathogens. Despite advances in understanding miRNA function, enigmas remain around miRNA regulation. Evidence is now emerging that extracellular matrices (ECM) molecules regulate miRNA expression. Here, we are developing a physiological, 3D matrix model that can be used as a substrate for macrophage culture and gene expression profiling.

Materials and methods

Human skin/foreskin fibroblasts isolated from a healthy adult male were used to synthesise and deposit ECM. ECM deposition and assembly was characterised by confocal and bright field microscopy, immunofluorescence, western blot and SDS-PAGE colloidal blue staining. Compatibility of matrices with primary human monocyte differentiation and culture was assessed by FACS analysis, MTT assay, cell adhesion, ELISA and qPCR.

Results

Total protein content of soluble and insoluble ECM extracts, colloidal blue staining and western blot confirmed ECM molecule (collagen 1, collagen 3, fibronectin and tenascin-C) deposition and assembly into a matrix. Immunofluorescence confirmed collagen fibre and fibronectin fibril formation, and preservation of the ECM after efficient decellularization. Confocal Z-stack imaging of collagen 1 and fibronectin allowed calculation of the ECM thickness (~20 µm).

Monocyte differentiation into mature macrophages on these decellularised matrices was similar to that on standard cell culture plastic. However, macrophage adhesion was different, indicating distinct cell interactions. Moreover, macrophages cultured on matrices induced similar levels of inflammatory cytokines in response to LPS, compared to those cultured on plastic. However, macrophages cultured on matrices displayed lower basal expression levels of cytokines and more stringently regulated expression of LPS-responsive miRNAs.

Discussion

We have established a standardised method for ECM deposition and decellularisation that is compatible with primary human macrophage culture and functional analysis. Macrophages cultured on matrices exhibit much more physiological behaviour than those seeded on standard cell culture plastic indicating our matrices as a promising model to study how the ECM regulates macrophage gene expression.

Workshop 8: Matrix Mechanobiology

P162, Murine cruciate ligament pathology and mechanics during osteoarthritis development

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Introduction
Osteoarthritis (OA) is a multicomponent joint disease characterized by articular cartilage degeneration, but also affecting surrounding tissue. Of interest are the ligaments which have been closely linked to OA in humans and in OA animal models. Our goal was to study the pathological changes in the ligaments during disease progression in murine spontaneous and posttraumatic OA.

Materials and Methods
Histological sections were taken from three different OA models: STR/ort mice, CBA mice following non-invasive knee trauma3, and C57Bl/6 mice following DMM surgery. Samples were imaged for µCT and stained with Toluidine Blue. Immunohistochemistry was performed at different OA progression stages; markers included cartilage matrix (collagen type II, and sox9), ligament markers (SCXA) and small leucine-rich proteoglycans (asporin). Gait analysis was analysed to determine the range of knee flexion necessary for tensile testing. Mechanical testing of the anterior cruciate ligament (ACL) measured its viscoelastic properties using an Instron. A 3D model was also created using Mimics (Materialise) for further finite element modelling.

Results
Toluidine Blue staining showed changes in the ligaments that were consistent with endochondral ossification, including increased extracellular matrix staining, loss of fibre alignment, and cell hypertrophy near attachment sites. Immunohistochemistry demonstrated modification in collagen type II and sox9 expression in these hypertrophic regions. In the trauma model, collagen type II deposition occurred in the mid-ligament region along with sox9 expression. µCT of the joint space revealed an increase in mineralized tissue volume with increasing severity of OA grades in all models. Gait analysis showed knee flexion between 55-100 degrees. Lastly, mechanical testing of the ACL showed the expected viscoelastic behaviour, and elastic modulus was calculated.

Discussion
Overall, ligament pathology is affected during OA progression, and pathology changes are consistent with chondrogenesis and potentially endochondral ossification. The full extent of these changes in the ligament matrix along with the consequences to OA remains to be seen.

P163, Collagen metabolism within a cardiac fibroblasts culture is dependent on physical properties of the cell environment

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Introduction
Extracellular collagen bound by integrins, conveys mechanical forces to the fibroblasts and the signal is supposed to influence metabolism of this protein within the cells. The aim of the study is to assess whether various hardness of the cardiac fibroblasts environment may modify collagen metabolism. Involvement of integrin alfa2beta1 in collagen deposition regulation will be evaluated.

Materials and Methods
The cardiac fibroblasts were cultured on both soft (15 kNt/m^2) or hard (28 kNt/m^2) poliacrylamide gels. Effect of the integrin alfa2beta1 inhibitor - TCI-15 (10^{-7}M, 10^{-8}M), on collagen deposition was investigated. ITGA2-gene siliencing was induced by the use of ITGA2-siRNA. The effectiveness of this process was confirmed by flow cytometry, Western Blot and qPCR.

Results
Augmented level of collagen (Woessner method) and expression of type I procollagen alfa 1 chain (qPCR) was found within the culture from the soft gel. Elevated, Matrix Metalloproteinase-1 (MMP-1) and decreased levels of Tissue Inhibitor of Metalloproteinases-3 (TIMP-3) and TIMP-4 were found within fibroblasts cultured on soft gel. Levels of MMP-2, MMP-9, TIMP1, TIMP2 and type III procollagen alfa 1 chain gene expression were not modified. Increased level of mRNA and protein for alfa 2 integrin subunit and alfa2beta1 integrin density was detected in cells from soft gel. Elevation of total and phosphorylated Src kinase content on the fibroblasts from soft gel were observed (immunoenzymatic method). TCI-15 augmented intracellular collagen content in cells cultured on both soft and hard gels.

Discussion
Hardness of environment modify collagen metabolism within cardiac fibroblasts. Thus, investigated physical properties of the fibroblast environment may change type I procollagen alfa 1 chain gene expression and influence collagen catabolism. These processes alter collagen level. Changes of the hardnes of the cell environment results in modification of alfa2beta1 integrin receptors density that is accompanied by alterations of Src kinase level and activity. Integrin alfa2beta1 is responsible for inhibition of collagen synthesis.
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P164, Allysine in Tropoelastin Assembly

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Introduction
Elastin is a mammalian extracellular matrix protein that plays crucial structural, mechanical and signalling roles in tissue. The building block of elastin is its monomer, tropoelastin, which is known to self-assemble and give rise to mature elastin fibres. Prevalent cross-links between lysyl oxidase-modified lysine residues, termed allysines, of tropoelastin contribute to the overall durability, strength and structure of elastin. It is currently unclear as to whether allysines play a role during the assembly stage. This study sought to understand the contribution of allysine modifications to elastin assembly.

Materials and Methods
We undertook an in silico approach to investigate the shape and dynamics of tropoelastin. Classical molecular dynamics and replica exchange simulations were used to examine the effects of allysine modifications in tropoelastin monomers. The allysine modifications were made at residues identified by our previous in vitro cross-linking experiments, and are further supported by native elastin mass spectrometry data (Schmelzer, C. and Heinz, A., pers. comm.).

Results
Tropoelastin exhibited a wide variety of tertiary structures dependent on the locations of the modifications and were accompanied by transient salt bridges involving lysines and key negative residues identified in previous studies. Allysine modifications not only altered the mobility of the domains containing the modifications, but also changed the global motions of the molecules. The combination of changes in tertiary structure and domain mobility altered the solvent accessibility of particular cross-linking domains, including those that are known to occur in native elastin.

Discussion
Taken together, these results indicate that allysine modifications are able to drive substantial changes in tropoelastin monomers. Alterations in domain mobility, solvent accessibility and global motions imply a difference in assembly as indicated by previous point mutation studies. In conclusion, we propose that allysines play a greater role in elastin assembly than previously believed, by not only forming cross-linking between molecules, but by also changing the dynamics of a single tropoelastin molecule to facilitate allysine modifications at appropriate sites.

P165, ST: Mechano-regulation of miRNA-221, -222, -21 and -27: implications for articular cartilage homeostasis

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Introduction

microRNAs (miRs) are small non-coding molecules that regulate post-transcriptional target gene expression. Increasing evidence supports a role for epigenetic mechanisms in articular cartilage homeostasis and disease e.g. osteoarthritis (OA). Abnormal mechanical load is a primary risk factor for OA development; previously, miR-221, -222, -146a and -365 were found to be mechano-responsive in chondrocytes. This project aims to (i) examine the mechano-regulation of miRs in articular cartilage subjected to normal and high loads *in vitro*, (ii) validate mechanically-regulated miRs in an *in vivo* model of post-traumatic OA and (iii) identify their downstream targets that could uncouple cartilage homeostasis in OA.

Materials and Methods

Bovine full-depth articular cartilage explants were subjected to loads of 2.5MPa (normal) or 7MPa (high) (1Hz, 15 minutes) and analysed 24 hours post-cessation of load; unloaded explants served as controls. Mechanically-regulated miRs were identified using Next Generation Sequencing (The Genome Analysis Centre, Norwich, UK). Expression of identified miRs were quantified in a mouse model of post-traumatic OA (load-induced rupture of the anterior cruciate ligament). Downstream targets of mechanically-regulated miRs were verified using 3'-UTR luciferase activity assays.

Results

miR-221 and miR-222, previously shown to be mechano-sensitive, were significantly increased in response to a high (7MPa) load compared to unloaded explants; both miR-221 and -222 expression also increased with increasing magnitude of load i.e. 7MPa compared to 2.5MPa load. miR-21 and miR-27a, known to control genes essential in cartilage homeostasis and regulated in OA, were upregulated in response to 7MPa load compared to either unloaded or 2.5MPa load. Interestingly, expression of these miRs were also significantly elevated in the cartilage of the *in vivo* loading model at 7 days post-rupture. *TIMP3* (Tissue Inhibitor of Metalloproteinase 3) and *CPEB3* (Cytoplasmic Polyadenylation Element Binding Protein 3) have been identified as putative downstream targets of these mechanically-regulated miRs.

Discussion

Our results confirm miR-221 and -222 mechano-regulation and demonstrate the novel mechano-regulation of miR-21 and -27a both in *in vitro* and *in vivo* loading models, miRs known to be involved in OA. Furthermore, TIMP3 and CPEB3 are putative targets of these differentially-regulated miRs and may mediate downstream effects that can lead to alterations in tissue homeostasis and/or cartilage degradation.

P166, Mechanical forces determine the spatial and temporal organisation of essential collagens in the developing skeleton

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Introduction
Mechanical forces affect both collagen synthesis and skeletal development. However, the effects of an abnormal mechanical environment on the emergence of collagen organisation have not been described. We examine how abnormal muscle loading affects the structural organisation of collagen type I, II, III, V, VI and X in the developing mouse forelimb.

Materials and Methods
Mice without skeletal muscle were generated ($Pax3^{Spd-/+}$,aka Splotch delayed). Embryos were harvested, staged according to Theiler stages TS22, TS25 and TS27 (typically embryonic days 13.5, 15.5 and 17.5). Collagen distribution was studied with immunofluorescence and confocal microscopy on cryosections.

Results
Abnormal muscle loading affected collagen I distribution at TS22–TS25, with decreased expression in mineralised cartilage compared to controls, but this recovered by TS27. The mesh- like organisation of collagen II of the control cartilage was absent in TS25 mutants. While collagen II was gone from the TS25 control mineralized cartilage, it was still present at low levels in mutants. At TS22, collagen V was expressed throughout the diaphysis of controls but absent in mutants. Collagen V did not appear in mutants until TS25, and even then with milder expression than controls. Collagen VI organisation and fibre orientation, and the shape and height of the chondrons, were abnormal in the mutants between TS25–TS27. Finally, collagen X expression in the mid-diaphysis of the humerus was decreased by at least 15% in the mutants. Collagen III was the only collagen examined that was unaffected by lack of muscle forces.

Discussion
Fetal immobility had dramatic effects on all but one of the collagens examined. The most substantial changes observed were delays in the initial expression of collagen I, II and V, loss of folded structure of the pericellular matrix, reduction in the column length of growth plate chondrons, changes in collagen VI fibre orientation in the mineralised cartilage and reduction in collagen X expression. Mechanoregulation of matrix may be playing a key role in the effects on skeletogenesis when mechanical stimulus is abnormal.

P167, ST: Extracellular matrix scaffolding: impact on vascular morphogenesis and endothelial mechanotransduction

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Introduction
Angiogenesis is associated with extensive extracellular matrix (ECM) remodeling. LOXL2 belongs to the lysyl oxidase family of proteins involved in collagen and elastin crosslinking. We previously showed that LOXL2 is required for formation of intersomitic vessels in zebrafish, and for capillary formation in vitro. We also found that LOXL2 colocalizes with collagen IV in the vascular basement membrane. We thus hypothesized that LOXL2 regulates angiogenesis and endothelial mechanotransduction through scaffolding of the ECM.

Materials and Methods
Interactions between LOXL2 and collagen IV were analyzed using proximity ligation assay and surface plasmon resonance. LOXL2-depleted and Col4a1-depleted human endothelial cells were generated to investigate ECM deposition. Mechanical properties of ECM and cells were probed by atomic force microscopy. Using 2D and 3D culture models, we tested the role of LOXL2 in vascular morphogenesis.

Results
We detected direct interactions between LOXL2 and collagen IV both intracellularly and in the ECM. Time-lapse TIRF microscopy demonstrated that LOXL2 is directly incorporated in ECM filamentous structures upon exocytosis. Indeed, LOXL2-depletion in endothelial cells altered deposition of ECM proteins, and decreased ECM stiffness, thus suggesting that LOXL2 regulates ECM scaffolding. We also investigated ECM deposition by Col4a1-depleted endothelial cells and found similar results as with LOXL2-depleted cells. Coating culture support with ECM proteins as collagen I or fibronectin was able to compensate for LOXL2 or Col4a1 depletion, suggesting a context-dependence of matrix scaffolding by endothelial cells. Formation of focal adhesions and cell contractility in response to ECM stiffness were not affected by LOXL2 depletion. Maturation of focal adhesions into fibrillar adhesions was however altered, as a result of defective ECM generation. Finally, whereas LOXL2-depleted cells organized in a network of discontinuous and thinner capillaries in a 2D cell-sheet co-culture set-up, these cells failed to generate capillaries in 3D hydrogels. Increasing the stiffness of hydrogels did not compensate for LOXL2 depletion.

Discussion
Our data demonstrate that LOXL2 regulates mechanotransduction in endothelial cells through scaffolding of the ECM. The molecular mechanisms involved in the maturation of structures controlling mechanosensing in response to ECM deposition are currently under investigation.

P168, Mesoscopic rigid body approach Modeling to study the dynamics of the ExtraCellular Matrix

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Introduction
The ExtraCellular Matrix (ECM) plays an important role in supporting tissue and organs, and has a functional role in the regulation of cell activity. However, the overall precise structural organization of the ECM is not well known. Some biophysical technologies allow accurate observation of molecules but not in a full ECM environment at the macroscopic and nanoscopic scales. We develop numerical tools that will help us in the modelling and study of molecular systems at the mesoscopic scale.

Materials and Methods
We reconstruct the molecules involved in parts of the ECM by gathering data from the PDB and other structural data sources. These often don't give us precise structures but they give us rough idea of the scale and shapes of these huge macromolecules. The ECM proteins are modelled as dynamic chains of rigid bodies interacting together in a virtual sandbox made using unity3D game and physics engines along with rigid body dynamics. Virtual reality and haptic devices are integrated to the simulation in order to enable the user to interact with the scene in an immersive manner and to better understand the mechano-behavior of the matrix.

Results
We reconstruct the molecules involved in parts of the ECM by gathering data from the PDB and other structural data sources. These often don't give us precise structures but they give us rough idea of the scale and shapes of these huge macromolecules. The ECM proteins are modelled as dynamic chains of rigid bodies interacting together in a virtual sandbox made using unity3D game and physics engines along with rigid body dynamics. Virtual reality and haptic devices are integrated to the simulation in order to enable the user to interact with the scene in an immersive manner and to better understand the mechano-behavior of the matrix.

Discussion
Using these approaches, we have results showing how parameters such as fiber flexibility or the nature and number of interactions between macromolecules can induce different structures in the basal lamina and in its mechanics. Virtual reality provides an intuitive mean of navigating complex environments crowded with macromolecules allowing us to observe molecular behavior that will induce cellular behavior.
From the preliminary simulations, it appears that fibers entanglement is strongly linked to their flexibility. More rigid fibers tend to form reticular structures with less entanglement. We can set given interactions to happen during the simulations. For instance, the laminin can interact with the integrin through its integrin-binding region. We can set our models to interactively create a joint between the models of the laminin and integrin to reflect such interactions.

P169, The Role of Integrin $\alpha_v\beta_3$ in Osteocyte Mechanotransduction during Estrogen Deficiency

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Introduction
The expression of the mechanosensor, integrin $\alpha_v\beta_3$, is reduced in osteoporotic bone cells compared to controls. MLO-Y4 osteocytes experience altered mechanotransduction under estrogen deficiency and it is unknown whether this is associated with defective $\alpha_v\beta_3$ expression or signalling. The objectives of this study are to (1) investigate $\alpha_v\beta_3$ expression and spatial organisation in osteocytes during estrogen deficiency, and (2) establish whether altered responses of osteocytes under estrogen deficiency correlate to defective $\alpha_v\beta_3$ expression and functionality.

Materials and Methods
MLO-Y4 cells were cultured as follows: Ctrl (no added estradiol), E+ (10nM 17β-estradiol for 5 days), and Ew (10nM 17β-estradiol for 3 days and withdrawal for 2 days). Cells were cultured with/without 0.5μM IntegriSense750 ($\alpha_v\beta_3$ antagonist). Laminar oscillatory fluid flow of 1Pa at 0.5Hz was applied for 1hr. $\alpha_v\beta_3$ content was quantified using an ELISA. The location and quantity of $\alpha_v\beta_3$ and vinculin (focal-adhesions) was determined by immunocytochemistry.

Results
Estrogen withdrawal under static conditions led to lower cell and focal-adhesion area ($p<0.05$), compared to E+ cells. **Fluid shear stress** led to higher $\alpha_v\beta_3$ content ($p<0.05$) in all groups, compared to static counterparts, with $\alpha_v\beta_3$ blocking altering this response. **Fluid flow on Ew cells** had the highest $\alpha_v\beta_3$ levels ($p<0.05$), but $\alpha_v\beta_3$ did not localise at focal-adhesions sites. Cell morphologies were similar after treatment with the **$\alpha_v\beta_3$ antagonist** to the Ew group.

Discussion
This study has shown for the first time that cell and focal-adhesion area are reduced following estrogen withdrawal. These results suggest there are fewer functional focal-adhesion sites at which $\alpha_v\beta_3$ integrins localise to facilitate mechanotransduction. To further understand these results, we are analysing osteocyte mechanotransduction by measuring integrin signalling (FAK, p-FAK) and gene expression (COX-2, RANKL, OPG, SOST).

P170, Using Cryo-TEM to determine the structure of fibrillin microfibrils

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Introduction
Fibrillin-1 is an extracellular matrix glycoprotein which polymerises through interactions with its N and C-termini to form microfibrils. Microfibrils are a crucial component of elastic fibres where they act as a scaffold for the deposition of elastin in elastic tissues such aorta, skin and lung. Fibrillin microfibrils are also important regulators of tissue homeostasis through their ability to bind and sequester growth factors in the matrix. The importance of fibrillin microfibrils is highlighted by the fact mutations in fibrillin-1 cause a number of diseases termed fibrillinopathies such as Marfan syndrome and the Acromicric dysplasias. To better understand how mutations in fibrillin cause disease it is crucial to understand how these mutations affect the structure and assembly of the mature microfibril. So far the high resolution structures of some individual domains and short fragments of fibrillin-1 have been determined, but it is not fully understood how they are arranged into microfibrils and how these microfibrils are arranged into larger structures in tissues

Materials and Methods
Microfibrils were extracted from dissected bovine ciliary zonule tissue before being purified using size exclusion chromatography. Microfibrils were then adsorbed onto quantifoil 2/2 grids and plunge frozen using a FEI vitrobot. Frozen hydrated samples were imaged using a Titan Krios equipped with a Gatan K2 Summit direct detector.

Results and Discussion
The nanoscale structure of extracted bovine ciliary zonule microfibrils was determined using high resolution cryo-TEM and single particle reconstruction. As microfibrils are flexible along their length sub-models of the different fibrillin regions were modelled separately to overcome the heterogeneity caused by this flexiblility. Microfibrils have a hollow tube-like structure with well-defined bead, arm, interbead and shoulder regions. The bead region has a complex double layered structure with an interwoven core and ring structures. The arm region has four separate densities which are potentially formed from dimers of fibrillin molecules. These high resolution models have also allowed for the fitting of molecular models into the different microfibril regions which has increased our understanding of how fibrillin is arranged in a microfibril.

P171, ST: Structure and interactions of elastic fibre proteins ADAMTSL2 and ADAMTSL4

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Introduction
Members of A Disintegrin and Metalloprotease with Thrombospondin type 1 motifs-like (ADAMTSL) proteins are extracellular matrix molecules that have been implicated in elastic fibre formation and play a role in fibrillin microfibril assembly and function. Mutations in ADAMTSLs phenotypically mimic genetic disorders caused by mutations in fibrillin-1 indicating that they contribute to common mechanisms in elastic fibre function. ADAMTSL2 interacts with fibrillin-1 and latent TGFβ binding protein-1 (LTBP-1) and mutations in *ADAMTSL2* give rise to geleophysic dysplasia. These data suggest that ADAMTSL2 may contribute to the structural maintenance of microfibrils and play a role in regulating TGFβ bioavailability. Furthermore, ADAMTSL4 has been associated with the structural arrangement of fibrillin-1 microfibrils in ciliary zonules of the eye and mutations in *ADAMTSL4* cause ectopia lentis. However, little is known about the structure and function of ADAMTSLs, except that they lack both the catalytic and disintegrin domains when compared to related ADAMTSs, ruling out a proteolytic function.

Materials and Methods
We have used negative-stain electron microscopy (EM), cryo-EM and small-angle X-ray scattering (SAXS) to determine the structures of ADAMTSL2 and ADAMTSL4. Surface plasmon resonance (SPR) has enabled us to study the interactions of ADAMTSL2 with other matrix proteins. Immunofluorescence microscopy was used to visualise the deposition of ADAMTSL2 and ADAMTSL4 in matrix produced by human dermal fibroblasts (HDFs).

Results and Discussion
Having cloned and purified recombinant ADAMTSL2 from mammalian cell lines, we employed cryo-EM to generate the 3D structure of ADAMTSL2 which revealed an asymmetric lobular shape which was verified by SAXS. SPR showed interactions of ADAMTSL2 with fibronectin and fibrillin-1. Moreover, visualisation of ADAMTSL2 at the cell surface using immunofluorescence microscopy has shown distinct co-localisation with fibronectin, fibrillin-1 and LTBP-1 in HDFs. Structural analysis of ADAMTSL4 using negative-stain EM has revealed that, unlike ADAMTSL2, it adopts several conformations suggesting it is highly flexible. We also observed complete co-localisation of the fibrous network of ADAMTSL4 with fibrillin-1 and partial co-localisation with LTBP-1 and fibronectin in HDFs.
Our investigation reports the first structural data on ADAMTSL molecules and furthers our knowledge of their biomolecular interactions providing a better understanding of their function in elastic fibre biology.

P172, ST: Nuclear decoupling is part of a rapid protein-level cellular response to high-intensity mechanical loading

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Introduction
Mesenchymal stem cells (MSCs) have a well-characterised mechano-response, including mechano-sensitive commitment to lineage. Cells from mature tissues must also respond appropriately to the mechanics of their surroundings, with cells in stiff and mechanically stressed environments requiring more robust cellular structures. However, many musculoskeletal disorders and connective tissue pathologies begin at sites of aberrant mechanical loading, suggesting a link between high mechanical stress and musculoskeletal disease. Furthermore, many tissues that experience complex mechanical loads, including heart and muscle tissue, have been proposed to benefit from MSC-based therapies. Whether MSCs have the means to survive and function correctly within high-intensity mechanical strain environments remains unknown. We aimed to understand how molecular processes within MSCs are affected by mechanical stress, and how cells maintain tissue homeostasis in response to elevated mechanical loads.

Materials and Methods
Human MSCs (n ≥ 3) adhered to type-I collagen-coated silicone membranes were dynamically strained (FlexCell system; low intensity 0 – 4% strain at 1 Hz and high intensity 2.6 – 6.2% strain at 5 Hz) for 1 hour, followed by a period of recovery. Cells were then analysed using high-content imaging and quantitative –omics technologies (transcript and protein), with and without perturbations to SUN2 expression levels.

Results
At low-intensity strain, morphological changes mimicked responses to increased substrate stiffness. As the strain regime was intensified we characterised rapid establishment of a broad, structured and reversible protein-level response, even as transcription was apparently downregulated. Protein abundance was quantified coincident with changes to protein conformation and post transcriptional modification. Furthermore, we characterised changes within the linker of nucleo- and cytoskeleton (LINC) complex of proteins that bridges the nuclear envelope, and specifically to levels of SUN domain-containing protein 2 (SUN2).

Discussion
Regulation of the LINC complex decouples mechano-transmission between the cytoskeleton and the nucleus, thus conferring protection to chromatin.

P173, Integrin α2β1 mediated structural differences in murine tendon

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Introduction
Integrins are a family of transmembrane proteins responsible for cell adhesion, substrate recognition and cross-talk between cell and extra-cellular matrices. Integrin α2β1 is a major receptor for monomeric collagen type I. Thus, it may play an important role in regulating the quantity and state of extracellular matrix. We could show that integrin α2β1 leads to increased quantities of collagen type I in bone. In this study we investigated the influence of the deletion of integrin α2β1 in tendons representing another collagen type I-rich musculoskeletal tissue.

Materials and Methods
Tenocyte-like cells harvested from murine achilles tendons were obtained and cultivated. The expression of type I and III collagen and scleraxis was analyzed by quantitative real-time PCR. Tenocyte cell culture matrix and tendon samples were quantified with the total collagen kit (quickzyme) by the hydoxyproline concentration. Transversal micro slices of tendon were prepared for histology and electron microscopy. Fibril diameter of the tendon resident collagen has been measured.

Results
Cell culture: Scleraxis and both collagens appear to be up-regulated on mRNA level. The upregulation of fibrillary collagens translates directly to higher collagen quantities measured in the cell culture lysates.
In-Situ: The number of tenocyte-like cells embedded in the extracellular matrix is not different. In contrary to the cell culture experiments, the overall collagen concentration in the integrin α2β1 deficient tendon in-situ is as high as in wild type tendons. However, on electron microscopy level integrin α2β1 deficient tendons show obvious changes to the wild type but this phenotype differs greatly form the one observed in bone. Whereas in bone collagenous fibrils appear to be amorphous and fused, the tendon-resident fibrils are quite distinct but interestingly much lower in mean diameter.

Discussion
The isolated tenocytes produce more collagen which is similar to the phenotype of bone cells. In-situ however, integrin α2β1 deficient tendons don't appear to contain more matrix, this may point to an altered turnover in the tendon. This hypothesis is supported by the appearance of more low-diameter collagen fibrils which may be newly formed. Such observations in the molecular structure impact the biomechanical properties of the integrin α2β1 deficient tendon.

P174, Cellular mechano-environment regulates the mammary circadian clock

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Introduction:
Circadian clocks drive ~24 h rhythms in tissue physiology. They rely on transcriptional/translational feedback loops driven by interacting networks of clock complexes. However, little is known about how cell-intrinsic circadian clocks sense and respond to their microenvironment.

Materials and Methods
Time-series microarray analysis was performed using mammary gland tissues isolated from C57BL/6 mice every 4 hours over 48 hours. Primary mammary epithelial cells were isolated from PER2::LUC reporter mice, and an in vitro 3D culture model was established to investigate the cell-autonomous mammary clock. The mammary stem cell self-renewal capacity was investigated using mammosphere assay in either WT or ClockΔ19 mutant mice.

Results
We identified a functional clock in mammary gland tissue, which rhythmically regulates the expression of ~600 genes with a period of 24 hr. The mammary clock is controlled by the cellular microenvironment in primary cell culture and in vivo. This is consistence with a dampened circadian rhythm observed in aged mammary tissue with stiffened matrix. Mechanistically, the tension sensing cell-matrix adhesion molecule, vinculin, and the Rho/ROCK pathway, which transduces signals provided by extracellular stiffness into cells, regulate the activity of the core circadian clock complex. We also show that genetic perturbation, or age-associated disruption of self-sustained clocks compromises the self-renewal capacity of mammary stem cells.

Discussion
Our work has revealed, for the first time, that circadian clocks are mechano-sensitive. The mammary clock is regulated by cell-matrix interaction, which provides a potential mechanism to explain how ageing influences clock amplitude and function. Furthermore, tissue stiffening suppresses the mammary circadian clock activity in vivo, which could contribute to an increased risk for breast diseases and even cancer.

P175, DOGME: towards the improvement of the atomic and molecular modelling of ECM proteins and components

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Introduction
Among the post translational modifications, N-glycosylations play an important role in the protein functions and some alterations of glycosylation (sialic-acid hydrolysis) can alter some protein activities. Considering their biological importance, it is essential to study glycosylated proteins. However, *in vitro* study of N-glycans remains very difficult because of the structural diversity and the many reactive groups of the glycan chains. Molecular Dynamics (MD) can then become a useful tool to overcome this problem and give access to conformational information through exhaustive sampling.

Materials and Methods
The research project we have been developing over the past two years is focused on the *in silico* DevelOpments for the study of Glycosylation and post-translational modifications applied to Extracellular Matrix proteins (DOGME project). Through this work, we want to elaborate a protocol that will allow any user to achieve in a single workflow, the building of a glycan structure, the launch of multiple and simultaneous MD simulations, the analysis and finally a state of the art visualisation of the generated trajectories. The main tools and softwares we have been using are the GROMACS MD software, the online tool CHARMM-GUI and the UnityMol molecular editor and viewer.

Results
The different developments we could address so far in the context of the DOGME projects are related to the following steps of the process: (a) building of a relevant N-glycan library, (b) elaboration of a program identifying the possible N-glycosylation sites and adding on the protein the selected modifications and (c) optimization of a visualisation tool allowing both experimentalists and modelling scientists to utilise the *in silico* results in a comprehensive and illustrative way. We have been testing and applying our tools on two types of ECM components: four human small leucine rich proteoglycans (SLRPs) (step b) and the Insulin Receptor (IR) which is implicated in type 2 diabetes (step c).

Discussion
The positioning of N-glycosylations on human SLRPs allow us to understand *visually* how much these modifications might impact the dynamic and behaviour of these proteins. In addition, the analysis of MD simulations of N-glycosylated IR show that our improved visualisation methodology has the potential to give informative results that could also help design in vitro experiments.

P176, Collagen crimp in the ageing human optic nerve head

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Introduction
Primary open angle glaucoma (POAG) is the second most common cause of irreversible blindness worldwide. The decreased mechanical compliance of the optic nerve head (ONH) with age may contribute towards the increased susceptibility of the elderly ONH to the development of POAG optic neuropathy. Therefore, this study aimed to quantify ONH collagen crimp as a function of age to investigate its potential role in ONH biomechanics.

Materials and Methods
Image datasets acquired from an age range of human ONHs (n=9, aged 0-30years, 31-60years and 61-88years) and glaucomatous (n=3) cryosections were excited at 800nm/12 and forward scattered second harmonic generation (SHG) signals was collected using a wavelength ultra-fast tuneable 140 fs mode-locked laser scanning microscope (LSM880 NLO with AxioExaminer^{TM} stand, Carl Zeiss Ltd, UK). Crimp period (CP), crimp amplitude (C_{Amp}) and degree of crimp were quantified in image datasets acquired from the lamina cribrosa (LC) and peripapillary sclera (ppsclera).

Results
Fibrillar collagen bundles in OHN from individuals <10 years of age had a wavy appearance which differs from that observed in older ONH (above the age of 40years). However, C_{Amp} in ppsclera (p=0.122) and LC (p=0.444) and degree of crimp in ppsclera (p=0.170) and LC (p=0.546) did not change with age. Ppsclera CP increased significantly from 19.64µm and 18.54µm in the 2 and 6-year-old, respectively to 22.04µm in the 88-year-old (rs=0.850, p=0.004). Additionally, LC CP was significantly greater in two of the three glaucoma ONHs, compared to age-matched controls (p=0.000). No differences were found in the C_{Amp}(p=0.139)and degree of crimp (p=0.723) in the glaucomatous LC and ppsclera.

Discussion
The changes in collagen crimp parameters within the ppsclera and LC as a function of age and/or glaucoma will influence ONH mechanics in response to intraocular pressure (IOP) elevation, and thereby effect mechano-transduction of cells within the LC.

P177, Perineuronal Chondroitin Sulfates Regulate Excitation/Inhibition Balance in Developing Vestibular Circuitry

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Introduction
Perineuronal nets (PN) are implicated in restricting neural plasticity with the maturation of neural circuits. Our study of the central vestibular nucleus (VN) found consolidation of PN around GABAergic interneurons as from postnatal day (P)9 of Sprague Dawley (SD) rats. Cleavage of chondroitin sulfates (CS) chains by microinjection of chondroitinase ABC (ChABC) resulted in delay of PN formation from P9 to P13. We hypothesized the delay would affect the excitation/inhibition balance in developing vestibular circuitry and acquisition of vestibular-dependent behaviours.

Materials and Methods
To find if cleavage of PN-CS by ChABC injection impacts on inhibition and excitation circuitry in VN during development, whole-cell patch-clamp recordings of spontaneous inhibitory and excitatory post-synaptic current (sIPSC and sEPSC) from VN interneurons were performed in brainstem slice preparations of P9 and P14 rats following test treatment with ChABC in the VN of P6 rats.
To find if PN-CS and PN-associated Semaphorin 3A bears behavioural consequences, the rats were assessed for developmental emergence of air-righting as read-outs of the maturation of the circuit for graviception.

Results
Significant reduction of sIPSC frequency and enhancement of sEPSC amplitude indicated disruption of PN-CS interfere the balance of excitation and inhibition in the circuitry.
Progressive localization of plasticity-inducing factor, Sema3A, to PN-CS was observed from P9 in VN. ChABC/Sema3A treatment of the rat VN at P6 resulted in delayed display of air-righting.

Discussion
CS disruption triggered plasticity at excitatory and inhibitory synapses onto otherwise PN-enwrapped neurons in the VN circuit. The behavioural readouts provide evidence for the role of PN-CS-Sema3A in controlling structural and circuit plasticity at the interneuron level with impacts on the developmental display of graviceptive behaviour.
Acknowledgement: The project is supported by general research fund (RGC-GRF 17125115).

P178, Cell mechanical response to growth factors and cross-linking in human engineered tendon constructs

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Introduction
Tendons transmit forces and maintain an internal tension that is a reciprocal relation of cell-generated forces and matrix properties. The mechanical forces are dependent on tissue properties and can be regulated by growth factors. The purpose of this study was to characterize the functional properties of human engineered constructs in defined mechanical alterations.

Materials and Methods
Human tendon fibroblasts within a fibrin scaffold gradually created their own matrix, which structurally resembles *in vivo* tissue. We manipulated matrix stiffness by a natural cross linker, genipin and also compared force responses with IGF-1 and TGF-b treatment. A custom-made force monitor was used which can apply force in a stepped manner and measure the outcome force at the same time, in culture conditions. The engineered tissues were subjected to cyclic unloading-loading protocols.

Results
When unloading the construct, cell re-tension was observed during the rest period, which is a tensional homeostatic response. Following a loading step, matrix relaxation was measured, which is due to viscoelastic properties of the constructs. The cell re-tension of genipin treated constructs was reduced in a dose dependent manner while the stress relaxation followed the same pattern. In contrast, genipin enhanced the peak modulus only at the highest concentration and the stiffness only at medium and high concentrations. BAPN treatment used as a negative control to prevent cross-link formation and confirmed by reducing dramatically the stiffness and the peak modulus whereas it had no effect on cell re-tension and matrix relaxation. Treatment with IGF-1 did not alter functional properties of the constructs whereas with TGF-b addition the cell re-tension increased compared with the untreated controls and IGF-1 treated. The matrix relaxation reduced only by TGF-b treatment.

Discussion
Disruption of tensional homeostasis eventually can lead to pathological conditions. These data provide useful information that may lead to specific targets regarding tissue healing or regeneration.

P179, The synovial surface of the articular cartilage. Ultrastructural aspects

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Introduction
Articular cartilage has been for long time the subject of intense research because of its evident clinical relevance. The composition and architecture of its matrix have been deeply investigated while its articular (synovial) surface, by contrast, has been the subject of few and sparse studies even it is obviously the only portion directly involved in the articular function. The present research represents a preliminary approach to an ultrastructural study on the articular surface.

Materials and Methods
Fragments of bovine articular cartilage were observed either unfixed by Fluid TM-AFM or briefly fixed in 1% Karnowski, dehydrated in graded ethanol and Hexamethyldisilazane (HMDS) and observed by SEM. Other fragments were fixed in Cupromeronic Blue (CB) or in Ruthenium Hexammine Trihydrate (RHT), dehydrated and observed by SEM. Finally, other samples were fixed overnight in 1% Karnowski, treated for 5 days with 2N NaOH and dehydrated and observed as above.

Results
In all cases the articular surface was made of thin, parallel fibrils immersed in a great amount of glycoconjugates, as confirmed by Fluid TM-AFM, CB and RHT. Treatment with concentrated NaOH completely removed all non-collagenic material and revealed a continuous surface of very thin (approx. 15 nm), uniform fibrils with various orientations depending from the sampling site. There were occasional evidences of a layered arrangement. Neither chondrocytes nor empty lacunae were detectable on the articular surface. The subsurface matrix down to the subchondral bone exhibited sparse normal chondrocytic lacunae and larger, heterogeneous collagen fibrils with diameters up to 150 nm.

Discussion
The free (synovial) surface of the articular cartilage is devoid of cells and composed of a uniform population of very thin, very regular collagen fibrils interconnected by copious proteoglycans. The fibrils are similar to those of the pericellular matrix and quite different from the larger, heterogeneous fibrils of the interterritorial matrix. It can be hypothesized that the articular surface is a sort of immature layer continuously deposed from the underlying chondrocytes, and that it can have functional properties different from the sub-surface matrix. The research is still underway.

P180. Cadherin-1 and cadherin-3 differentially regulate pancreatic cancer cell invasion through type I collagen

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Introduction:

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers in the world, particularly due to its late diagnosis and its resistance to chemotherapeutic agents. The complexity of this pathology is implemented by a dense desmoplastic stroma with type I collagen as a major component, an expansive tissue invasion and early metastasis. Interestingly, alterations in the expression of cell adhesion molecules including cadherins and integrins have been reported in PDAC. Yet, how these changes contribute to tumour progression is poorly understood. Here, we investigated the role of cadherins during PDAC progression.

Material & Methods:

Cadherins expression was immunohistochemically examined through human pancreatic cancer development and in PDX1-Cre, $Kras^{G12D}$, $Ink4a/Arf^{flox/flox}$ mouse model developing PanIN lesions similar to human ones. To analyze a crosstalk between cadherins during PDAC progression, we selected BxPC-3 pancreatic cancer cell line expressing both cadherin-1 and -3 and deleted by shRNA strategy only one of them. The roles of each cadherin in cell invasiveness were assessed by using in vitro (2D and 3D invasion, invadopodia assay, ...) and in vivo approach.

Results:

Double labelling experiments revealed that cadherin-3 is early detected at the plasma membrane during progression of pancreatic intraepithelial neoplasia 1 (PanIN-1) to PDAC, suggesting that this molecule could be an early marker for this pathology. Despite tumoral cells turn on cadherin-3, significant amount of cadherin-1 remains associated to the cell membrane.

Orthotopic and ectopic injections in nude mice of these cell lines demonstrated that cadherin-3 mainly regulates tumor growth. However cadherin-1 drives type I collagen fibers organization in the tumor. Indeed, cadherin-1 depletion strongly reduced the presence of collagen fibers in the stroma. These observations lead us to analyze the role of cadherins in cell invasiveness. We showed that cadherins differentially participate to cell invasion and migration: cadherin-3 regulates collective cell migration whereas cadherin-1 takes part to the modulation of gelatinolytic activity. These results were confirmed by using PDAC-derived primary cell cultures issued from patient–derived xenograft in nude mice.

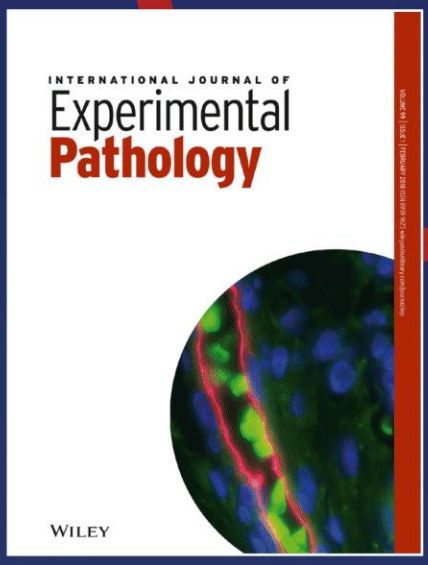
Discussion:

Both cadherin-1 and cadherin-3 differentially induce PDAC aggressiveness. This should improve our understanding of PDAC in order to identify new efficient therapeutic targets.

Sponsor Flyers and additional Workshops

International Journal of Experimental Pathology

is proud to sponsor the Fell Muir Prize, awarded by the British Society of Matrix Biology.



The Fell Muir Prize has been awarded to scientists of international stature, and who demonstrate outstanding achievement in the field of matrix biology, since 2006.

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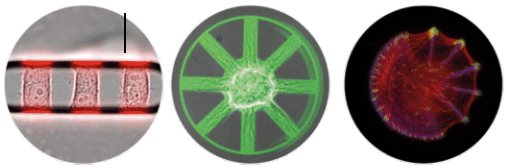
WILEY

WORKSHOP ANNOUNCEMENT FROM ALVÉOLE IN COLLABORATION WITH
THE UNIVERSITY OF MANCHESTER & THE WELCOME TRUST CENTRE FOR CELL MATRIX RESEARCH



Control cell microenvironment topography and biochemistry with custom, contactless, maskless photopatterning

Tuesday 24 July 2018
Manchester, UK

PROGRAMME

14:00 – 18:00

- Presentation of PRIMO custom photopatterning technology & applications
- Demonstration of 2D quantitative protein photopatterning & cell micropatterning
- Demonstration of microfabrication of UV-sensitive resist
- Hands-on session for the participants to try the technology

SPEAKERS

Dr Mehmet Deniz Akyüz – Business Development Manager Europe, Alvéole
Dr Grégoire Peyret – Application Engineer, Alvéole

IN COLLABORATION WITH

Dr Christoph Ballestrem – Senior Lecturer, Division of Cell Matrix Biology and Regenerative Medicine
Aljona Kolmogorova – PhD Student, Division of Cell Matrix Biology and Regenerative Medicine
Dr Peter March – Senior Experimental Officer, Bioimaging Facility

WORKSHOP VENUE

University of Manchester
Michael Smith Building, B3011 - Oxford Road
Manchester, M139PT

PREVIOUS REGISTRATION NEEDED FOR PARTICIPATION

Contact: Dr Mehmet Deniz Akyüz,
mehmet@alveolelab.com

MANCHESTER 1824 The University of Manchester

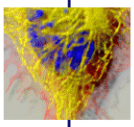
wellcome centre cell-matrix research

alvéole ALVEOLELAB.COM

Specification Sheet Huygens Software

Scientific Volume Imaging has a long-standing experience in high-quality deconvolution and is well-known for its Huygens software. Users of Huygens are offered specialized algorithms to restore and improve confocal, widefield, spinning disc, multi-photon, STED 3D, and SPIM/Light Sheet image data. Data can range from 2D images to extreme large datasets of 32 channel 3D-time series.

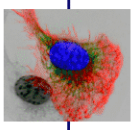
Huygens combines restoration with advanced visualization and analysis features for producing high quality and publishable results. Titan proves that image organization, searching, and annotation is extremely easy and does not require maintaining a image database, or moving or editing precious data. Huygens products use CPU and GPU-acceleration optimally, and are very well suited for batch and high-throughput deconvolution.



Platforms & System requirements

Huygens is available as 64-bit and multi-processor version for all platforms. All applications have the same look and feel on these platforms, except for the differences by the window manager of the operating system. Performance of all deconvolution algorithms is ensured with optimal processing on both GPU's (NVIDIA - CUDA based acceleration) and CPU's.

Huygens Volume's Renderers are based on rendering continuous iso-surfaces with fast ray tracers offering the highest possible quality of visualizing your image data.



Windows

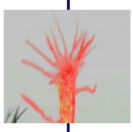
Available 64-bits versions for Windows Vista and up.

- Processor: AMD or Intel.
- Recommended RAM: 8 GB or more.

Apple Mac OS X

Mac OS X 10.7 and up is required for full 64 bit capabilities, and full multiprocessor support on Intel platforms.

- Processor: Intel.
- Recommended RAM: 8 GB or more.



Linux

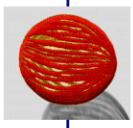
Linux RedHat, SuSE and Ubuntu are supported. Huygens works on many other distributions as well (64 bit).

- Processor: AMD or Intel.
- Recommended RAM: 8 GB or more.

Image file formats

Huygens is compatible with a variety of image formats:

- Reads/writes HDF5 (Hierarchical Data Format), ICS & ICS2 (Image Cytometry Standard), OME (Open Microscopy Environment), MRC (.r3d), Nikon-ICS, Leica style TIFF series, Olympus SIS Tiff Series, Biorad 'PIC', and Imaris classic images, AVI
- Reads Zeiss CZI, ZVI and LSM, Leica LIF and LOF, Metamorph STK and ND, Olympus OIF and VSI, BioVision IPM and IPL, Nikon ND2
- Reads/writes a single or numbered series TIFF images into/from 3D volume images, BigTIFF



More information about the options can be found at:
http://www.svi.nl/HuygensProducts

	Huygens Essential	Huygens Professional	Huygens Scripting	Huygens Core
64 bit (Windows, Mac, Linux)	√	√	√	√
1 to 6 cpu threads use	√	√	√	√
From 6cpu threads and up (option)	√	√	χ	√
GPU acceleration (NIVIDIA CUDA based)	√	√	√	√
Floating license set-up (option)	√	√	χ	χ
User Interface				
Compatible with **Titan** as a front-end for image indexing, searching and annotation (option)	√	√	χ	χ
Leica LAS AF/X data exchange (option)	√	√	χ	χ
Wizard-like User Interface	√	√	χ	χ
Extensive toolbox User Interface	χ	√	χ	χ
Tcl shell	χ	√	√	√
Web-browser interface (HRM)	χ	χ	χ	√
Schedulers for batch deconvolution				
Wizard-like Batch Processor	√	√	χ	χ
Tcl/Tk based automation	χ	√	√	√
HRM, web-based interface for automation	χ	χ	χ	√
Optical Options				
Widefield/Brightfield, confocal, multi-photon, spinning disc (options)	√	√	√	√
STED and STED 3X (option)	√	√	√	√
SPIM/Light Sheet (option)	√	√	χ	√
File format compatibility				
2D, 3D, multi-channel images and directories	√	√	√	√
Time series (option)	√	√	√	√
Reads hdf5, ics, ome, tiff (also Olympus), Bio-rad PIC, r3d, dv, Imaris classic	√	√	√	√
Writes hdf5, ics, ome, tiff, Biorad PIC, r3d, Imaris classic, avi	√	√	√	√
Reads Zeiss ZVI-LSM-CZI, Leica LIF-LOF, Olympus OIF, Olympus VSI, BioVision IPM/TIF, Nikon ND2, Metamorph STK, ND (option)	√	√	√	√
Multi-channel images				
Wizard for deconvolving images per channel	√	√	χ	χ
Automatic deconvolution	χ	√	√	√
Image report				
Histogram and image statistics	√	√	√	√
Distance measurements, Intensity Flux Plot	χ	√	√	√
Ratio image	χ	√	√	√
Image Preprocessing Methods				
(Automatic) cropping	√	√	√	√
Converting image dimensions	√	√	√	√
Invert image (brightfield)	√	√	√	√
Blacklevel adjustment	√	√	√	√
Mirror along XYZT	√	√	√	√
Shift, split and join channels	√	√	√	√
Re-sample, iso-sample, Copy and stretch	χ	√	√	√
Soft-threshold, soft-clip	χ	√	√	√
Add or remove border, insert frame/slice	χ	√	√	√
Filters	χ	√	√	√
Fourier Transforms	χ	√	√	√
Deconvolution algorithms				
Iterative,MLE, Classic MLE, Quick MLE	√	√	√	√
Good Roughness MLE	√	√	√	χ
Iterative TM, Quick TM	χ	√	√	√
Automated image correction				
Bleaching and lamp jitter correction	√	√	√	√
Spherical aberration correction	√	√	√	√
Coverslip positioner for setting image depth	√	√	√	√
Z-drift correction (option)	√	√	√	√
Image Correction				
Hot Pixel Remover	√	√	χ	χ
Chromatic Aberration Corrector (option)	√	√	χ	√
Crosstalk Correction (option)	√	√	χ	χ
Object Stabilizer (option)	√	√	χ	√
Stitcher (option)	√	√	χ	χ
PSF Distiller				
Theoretical PSF is always made	√	√	√	√
Experimental PSF is used	√	√	√	√
Experimental PSF measurement per channel	χ	√	√	√
Experimental PSF distiller (option)	√	√	χ	χ
Visualization				
Twin Slicer	√	√	χ	χ
Slicer	χ	√	χ	χ
Orthogonal Slicer	√	√	χ	χ
Simulated Fluorescence Process Renderer	√	√	χ	χ
Maximum Intensity Projection Renderer	√	√	χ	χ
Surface Renderer (option)	√	√	χ	χ
Movie Maker (option	√	√	χ	χ
Image / Object Analysis				
Object Tracking (option)	√	√	χ	χ
Colocalization (option)	√	√	χ	√
Advanced Object Analysis (option)	√	√	χ	χ
Other features				
Display of deconvolved images in progress	√	√	χ	χ
Simulation of Microscopy Image Formation	χ	√	√	√
Templates for microscopy and deconvolution parameters can be saved and re-used	√	√	√	√

Scientific Volume Imaging b.v.
Laapersveld, 63. 1213 VB Hilversum. The Netherlands.
tel.: +31 35 642 16 26 fax: +31 35 683 79 71
email: info@svi.nl URL: http://www.svi.nl

On-line specification sheet at
www.svi.nl/HuygensSpecifications

Push the Frontiers of Resolution!

Improve resolution up to 4 times and increase SNR more than 10 times!

Huygens Software

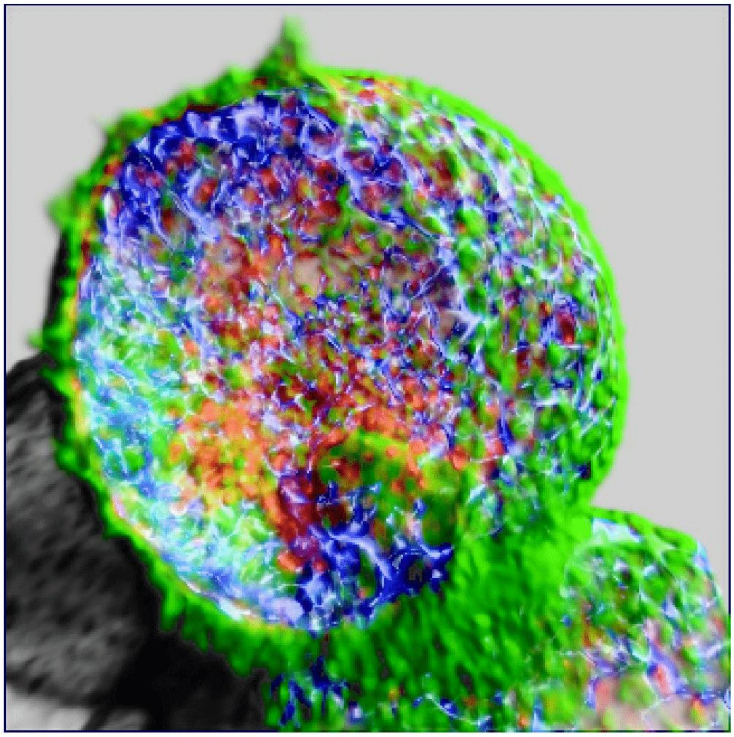
Specification Sheet

Huygens Essential
Huygens Professional
Huygens Scripting
Huygens Core

Windows • Mac OS X • Linux

Scientific Volume Imaging b.v.

Huygens Software



High quality deconvolution, restoration, visualization and analysis

Confocal • Widefield • Multi photon • Spinning disk
(3D) STED • Light Sheet (SPIM) • Floating Licenses
GPU enhanced Performance • PSF distiller
Chromatic Aberration Corrector • Volume and Surface
Renderers • Object & Colocalization Analyzers
Tracking • Stitching • Organizing and Searching images
From 2D to 3D multichannel and time lapse data

Windows • Mac OS X • Linux

Scientific Volume Imaging b.v.

Huygens Software

Deconvolution - Visualization - Analysis

Huygens Essential guides you step-by-step through the deconvolution process, making deconvolution as simple as possible without sacrificing quality. The advanced **Huygens Professional** provides you with a choice of algorithms and tools to restore your images. Both Essential and Professional include a Batch Processor.
Huygens Scripting enables you to process large number of images using scripts and a command-line interface. **Huygens Core** has powerful multi user batch deconvolution and can be integrated in web applications for online image processing. **Huygens Titan** helps searching and indexing your images data. The **Huygens Floating License** option provides an economical and flexible solution for multiple users.

Huygens deconvolves confocal, wide-field, spinning disc, multiphoton, STED and Light Sheet/SPIM image data. Includes powerful visualization tools, such as the Twin Slicer, Orthogonal Slicer, 3D Maximum Intensity Projection (MIP) and Simulated Fluorescence Process (SFP) renderer. The following options can be added to your Huygens basics:

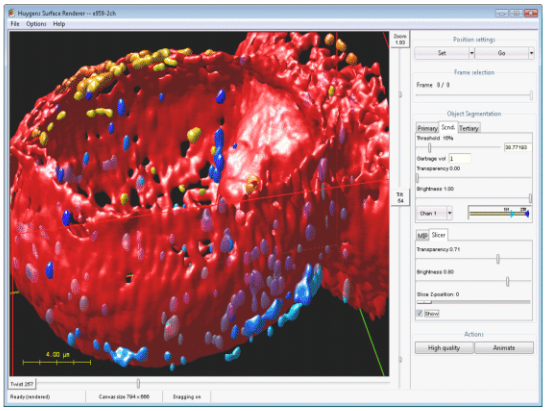
Multi-GPU enhanced performance for optimized deconvolution and other FFT-based operations.

The PSF distiller is a wizard for measuring your microscopes Point Spread Functions.

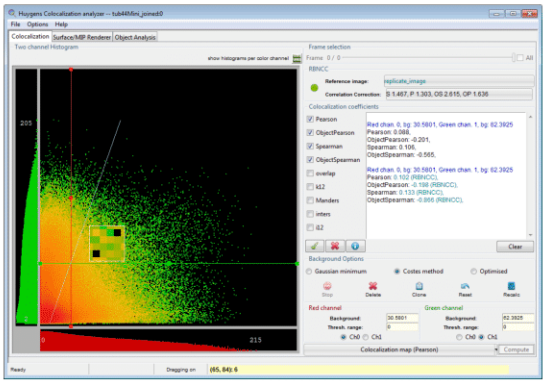
Extended File Reader supports Zeiss ZVI, LSM, CZI, Leica LIF and LOF, Olympus OIF and VSI, MetaMorph STK and ND, BioVision IPL and IPM, Nikon ND2 file formats.

SPIM Fusion Wizard facilitates deconvolution, scatter correction, and fusion of multi view SPIM/Light Sheet data sets.

Surface Rendering of allows you to explore your 3D-5D data in high quality using fast ray tracers.

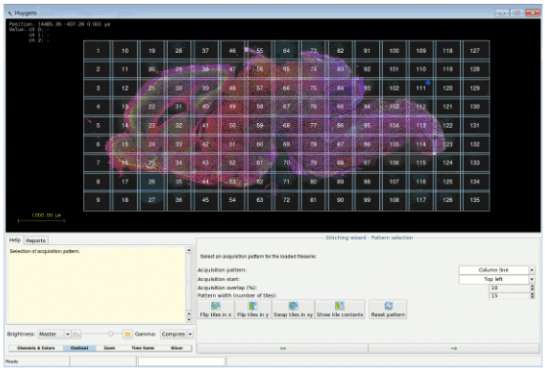


Colocalization Analyzer computes the well-known coefficients such as Pearson, Spearman, Overlap, and Manders M and K.



Movie Maker creates sophisticated combined animations from the MIP, SFP and surface renderer interfaces.

Stitcher Wizard stitches large datasets and tile numbers. Integrated deconvolution and automatic vignetting correction available.



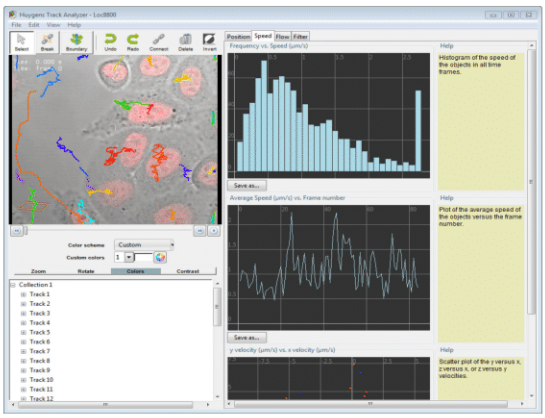
Chromatic Aberration Corrector automatically estimates and corrects for differences in shift, rotation, and magnification between channels.

Crosstalk Corrector detects and corrects for (linear) crosstalk between all channels in multi-channel images.

Object Analyzer is an interactive tool to obtain elaborate object statistics from 3D-5D images.

Object Stabilizer is a wizard-driven stabilizer for 2D or 3D time series, and slices within a 3D stack.

Object Tracker enables you to track objects through time, analyses the tracks, and provides you with detailed information about the movement of the objects.



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Free demo and on-line supports for Huygens Software available from **www.svi.nl**.

The 11th Asian and Pan-pacific Connective Tissue Society Symposium & The 3rd National Conference of CSMB

Nov 16th-20th, 2018

Hangzhou, Zhejiang Province, China

Organizer： Chinese Society of Matrix Biology (CSMB)

Co-organizers： Peking University

Zhejiang University

Chinese Society of Matrix Biology (CSMB), established on October 23, 2015, is an academic, non-profit organization affiliated to Chinese Association for Physiological Science (CAPS) and engaged in the dissemination of information relating to research on the extracellular matrix, cell-matrix interactions, and related areas in biology and medicine. The committee members consist of the matrix biologists and scientists majored in physiology, cell biology, pharmacology and clinical medicine from all over China.

Under the authorization of International Society for Matrix Biology (ISMB) to the Chinese Association for Physiological Sciences, CSMB will hold the 11th Asian and Pan-pacific Connective Tissue Societies Symposium and the 3rd Academic Annual Meeting of the CSMB on Nov 16th-20th, 2018 in Hangzhou International Expo Center, Zhejiang Province, China. This biennial symposium on connective tissue covers multiple fields including matrix biology, stem cell biology and applications, tissue microenvironment and cancer metastasis. This symposium will provide an excellent arena for fostering collaborations and creating sparks of thought. Most of the distinguished scientists in the field are invited to lecture and share their findings and educational experience to the young scholars. The organization committee welcomes all scientists in matrix biology or other related fields to participate in the conference to present and communicate their exciting research works.

1. Date and venue

Date： Nov 16th-20th, 2018 (Registration on Nov 16th, 2018; Conference Program during Nov 17th-20th, 2018)

Venue： Hangzhou International Expo Center (No.353 Benjing Avenue, Qianjiang Century City, Xiaoshan District, Hangzhou, Zhejiang, China)

2. Organization Committee

Honorary President:

Qimin Zhan (Director of Peking University Health Science Center， Chinese Academy of Engineering academicians)

President:

Hongquan Zhang (Peking University Health Science Center)

Vice President:

Wei Kong (Peking University Health Science Center), Chuanyue Wu (Southern University of Science and Technology), Zhigang Zhang (Shanghai Cancer Institute), Danny Chan (University of Hong Kong), Luyang Yu (Zhejiang University), Jianfeng Chen (Shanghai Institutes for Biological Sciences), Liliana Schaefer (President of The International Society for Matrix Biology)

Organizing committee:

Jianfeng Chen, Danny Chan, Hongbin Han, Wei Kong, Chuanyue Wu, Jianliu Wang, Luyang Yu, Hongquan Zhang, Ning Zhang, Zhigang Zhang, Weiguo Zhu

Domestic academic committee:

Huijie Bian, Zhijie Chang, Ceshi Chen, Jianguo Chen, Jianfeng Chen, Jianquan Chen, Wei Chen, Danny Chan, Hongkui Deng, Bisen Ding, Erdan Dong, Jie Du, Junbao Du, Weigang Fang, Bo Gao, Gaoxiang Ge, Mingzhou Guo, Youfei Guan, Zhuowei Hu, Bo Huang, Yu Huang, Qing Jiang, Wei Kong, Enyin Lai, Enmin Li, Xiaowei Li, Aifu Lin, Xinhua Lin, Baohua Liu, Zhihua Liu, Wen Ning, Jincai Luo , Gaoliang Ouyang, Xiaoyan Qiu, Jianliu Wang, Nanping Wang, Lin Wang, Xian Wang, Zimei Wang, Congyin Wu, Chuanyue Wu, Liling Wu, Congjian Xu, Liu Yang, Luyang Yu, Yanqiu Yu, Jun Zhan, Li Zhang, Lingqiang Zhang, Hongquan Zhang, Xiaoling Zhang, Yan Zhang, Youyi Zhang, Zhigang Zhang, Zhiqian Zhang, Qiang Zhao, Jingang Zheng, Junke Zheng, Jing Zhou, Zhongjun Zhou, Weiguo Zhu, Yi Zhu, Guanglei Zhuang, Xuenong Zou

International academic committee:

Liliana Schaefer, Suneel S. Apte, Lenna Bruckner, Alain Colige, Anthony J Day, Eok Soo Oh, Jianglin Fan, Reinhard Fässler, Junlin Guan, Donald Gullberg, Jyrki Heino, Renato V. Iozzo, Chuanju Liu, Qingjun Meng, Jun Qin, Francesco Ramirez, Lynn Sakai, Lydia Sorokin, Staffan Strömblad, Samuel I. Stupp, Hideto Watanabe, James Whiteford, John Whitelock

3. Abstract Submission

The conference includes Invited Talks, Oral Presentations and Poster Presentations. The Oral and Poster Presentation are currently open for abstract submission. To encourage the participation of young scholars, the organization committee will provide Travel Award for 6 young scholars selected by academic committee based on the submitted abstracts.

Abstract must be in English with less than 500 words and displayed in single-spaced with 11 point Font of Times New Roman. Please submit abstract before July 30, 2018 and define it for application of oral or poster presentations. For more detailed information of abstract submission, please go to http://www.pptcss2018.org/ or contact us through email csmb2016@163.com.

Requirements for the applicants of Travel Award:

1. The applicant should be either post-doc or graduate student under 40-year old (born after Jan 1st, 1978).
2. The applied work should be related to matrix biology area as basic, translational or methodology studies.
3. The applied work should be unpublished.
4. The applicant should address the application of “Travel Grant” in the abstract.

4. Registration Information

Category	Register and Pay before July, 31 2018 (Included)	Register and Pay from August, 1 2018 to November 10 2018	Register and Pay from November 11 2018 to November 18 2018	CAPS Membership Fee
Foreign Participants	3000 RMB	3500 RMB	4000 RMB	
Remark	Due to our financial policy, no reimbursement can be made for cancellation. Instead, replacement of participant is welcome.			

5. Registration

Please visit the symposium website (http://www.pptcss2018.org/) for Registration and Payment.

Enquiry email: csmb2016@163.com

6. Accommodation Information

Please visit the symposium website (http://www.pptcss2018.org/) for booking the rooms.

7. Invited Speakers

	Plenary talk Host: Hongquan Zhang			
1	Reinhard Fässler	Max Planck Institute of Biochemistry	Genetic and biochemical analysis of focal adhesion proteins	faessler@biochem.mpg.de
	Session 1, Novel aspects in matrix biology (technological and conceptual advances)			
2	Qingjun Meng	University of Manchester, UK	ECM-dependent circadian clocks in mammary epithelia: potential links to breast cancer	qing-jun.meng@manchester.ac.uk
3	Xiaowei Li	Shanghai Jiaotong University	Whole-mount tissue clearing and imaging with single-cell resolution	XL3A@sjtu.edu.cn
4	Alain Colige	University of Liege, Belgium	The functions of ADAMTS2, 3 and 14 extend well beyond their aminoprocollagen peptidases activity	acolige@uliege.be
5	Francesco Ramirez	Icahn School of Medicine at Mount Sinai	Computational approaches to identify new drug treatments of aneurysmal disease	francesco.ramirez@mssm.edu
	Session 2, Signaling From the Matrix			
6	Jianfeng Chen	Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences	Integrin a4b7 switches its ligand specificity via distinct conformer-specific activation	jfchen@sibcb.ac.cn
7	Chuanyue Wu	Southern Medical University	Integrin singaling and human diseases	carywu@pitt.edu
8	Jyrki Heino	Department of Biochemistry, University of Turku	Functional role of post-translational modifications in ECM proteins	jyheino@utu.fi
9	Junlin Guan	University of Cincinnati College of Medicine	Focal adhesion kinase: the first quarter century and beyond	guanjl@ucmail.uc.edu
10	Jun Qin	Baylor College of Medicine	Molecular insight into the regulation of integrin activation and focal adhesion assembly	jqin1965@126.com
11	**Session 3, Matrix in development and disease**			
	Xinhua Lin	Fudan University	Regulatory mechanisms of proteoglycans in endoderm patterning during development	Xlin@fudan.edu.cn

12	James Whiteford	Queen Mary University Of London	A novel role for Syndecan-4 in neovascular diseases	j.whiteford@qmul.ac.uk
13	Xuenong Zou	Institute of Orthopedics, the First Affiliated Hospital, Sun Yat-sen University	ECM and Intervertebral disc degeneration	zxnong@hotmail.com
14	Wei Kong	Peking University Health Science Center	ECM Regulation of Vascular Smooth Muscle Cell Identity	kongw@bjmu.edu.cn
15	John Whitelock	UNSW Sydney	The differential biological activities of the basement membrane heparan sulfate proteoglycan, perlecan in vascular, inflammatory and induced pluripotent stem cells	j.whitelock@unsw.edu.au
16	Lenna Bruckner	Dept. Dermatology, Medical Center - University of Freiburg	Translational skin matrix research: disease mechanisms and therapies	leena.bruckner-tuderman@uniklinik-freiburg.de
	Session 4, Matrix dynamics and turnover			
17	Suneel S. Apte,	American Chairman of Matrix Biology Society	Unexpected roles for metalloproteases in cell regulation	APTES@ccf.org
18	Hideto Watanabe	Japanese Chairman of Matrix Biology Society	Role of versican and its processing	wannabee@aichi-med-u.ac.jp
19	Anthony J Day	Faculty of Biology, Medicine & Health, The University of Manchester	Glycosaminoglycan reorganisation and crosslinking mechanisms in ovulation and inflammation	Anthony.Day@manchester.ac.uk
20	Jianglin Fan	University of Yamanashi	Functional roles of MMPs in the pathogenesis of atherosclerosis	jianglin@yamanashi.ac.jp
	Session 5, ECM in fibrosis and cancer			
21	Renato V. Iozzo	The editor of Matrix biology	Dual regulation of angiogenesis and autophagy by proteoglycans and its parts	Renato.Iozzo@jefferson.edu
22	Hongquan Zhang	Peking University Health Science Center	Kindlin-2 control fibrosis via multiple signaling pathway	Hongquan.zhang@bjmu.edu.cn
23	Lin Wang	The Fourth Military	Sinusoidal endothelial	fierywang@1

		Medical University	niche and liver fibrosis	63.com
24	Wen Ning	Nankai University	Role of matricellular protein Fstl1 in lung development and fibrosis	ningwen108@nankai.edu.cn
25	Zhuowei Hu	Institute of Materia Medica, Peking Union Medical College	Title to be determined	huzhuowei@imm.ac.cn
26	Bo Huang	Peking Union Medical College	Killing the soft matrix for cancer treatment	tjhuangbo@hotmail.com
	Session 6, ECM in Inflamation and Immunity			
27	Lydia Sorokin	Univerisity of Muenster, Germany	The Differential Role of the Endothelial Basement Membrane and the Gelatinases in Leukocyte Extravasation into the Brain	sorokin@uni-muenster.de
28	Liliana Schaefer	International Chairman of Matrix Biology Society	Advances in SLRP biology	schaefer@med.uni-frankfurt.de
29	Eok Soo Oh	Vice President, Office of Research President, University-Industry Collaboration Foundation EWHA WOMANS UNIVERSITY	Unique expression of syndecan-2 in proximal colon during acute inflammation	ohes@ewha.ac.kr
30	Chuanju Liu	New York University Medical Center	Interplay among ADAMTs and inflammatory cytokines in arthritis	chuanju.liu@nyumc.org
	Session 7, ECM in stem cell and regeneration			
31	Hongkui Deng	Peking University Health Science Center	Small molecules induced cell reprogramming	hongkui_deng@pku.edu.cn
32	Bi-Sen Ding	Weill Cornell Medicin, Sichuan University	Vascular MMP14 regulates organ regeneration and fibrosis	dingbisen@scu.edu.cn
33	Danny Chan	The University of Hong Kong	*Lgr5* and *Col22a1* mark differentiation of joint progenitor cells to juvenile articular chondrocytes	chand@hku.hk
34	Zhongjun Zhou	The University of Hong Kong	Isthmin is a novel inhibitor of nodal signaling	zhongjun@hku.hk
	Session 8, Mechanosensing			
35	Jing Zhou	Peking University	Transduction of mechanical	jzhou@bjmu.

		Health Science Center	cues by discoidin domain receptor 1 and DNA methyltransferase 1	edu.cn
36	Yu Huang	Cardiovascular Research Center , The Chinese University of Hong Kong	From skeleton to cytoskeleton: osteocalcin transforms vascular fibroblasts to myofibroblasts via angiotensin II and Toll-like receptor 4	yu-huang@cuhk.edu.hk
37	Congying Wu	Peking University Health Science Center	Gradient of matrix stiffness regulates cell migration and mitosis	emmacongyingwu@163.com
38	Samuel I. Stupp	Northwestern University	Supramolecular Engineering of Bioactive Extracellular Matrices	s-stupp@northwestern.edu
39	Donald Gullberg	University of Bergen	Title to be determined	donald.gullberg@uib.no
40	Aifu Lin	Zhejiang University	Title to be determined	linaifu@zju.edu.cn
41	Staffan Strömblad	Department of Biosciences and Nutrition, Clinical Molecular Biology Karolinska Institutet	Novel cell-matrix adhesion structures	Staffan.Stromblad@ki.se
42	Lynn Sakai	President of ASMB	Title to be determined	lys@shcc.org

The 11th Asian and Pan-Pacific Connective Tissue Societies Symposium (PPCTSS) & The 3rd National Conference of CSMB

Time: Nov 16-20, 2018
Venue: Hangzhou International Expo Center, Hangzhou, China

Theme: *Connective Tissue: Environment of cell life activities-basic and clinical applications*

Topic:

- **Session 1** Novel aspects in matrix biology (technological and conceptual advances)
- **Session 2** Signaling from the matrix
- **Session 3** Matrix in development and disease
- **Session 4** Matrix dynamics and turnover
- **Session 5** ECM in fibrosis and cancer
- **Session 6** ECM in inflammation and immunity
- **Session 7** ECM in stem cell and regeneration
- **Session 8** Connective tissue and mechanosensing

Registration website: http://pptcss2018.org

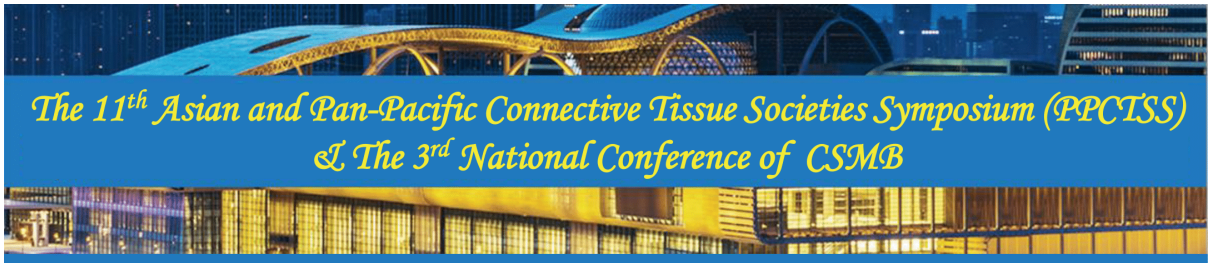
Organized by:

- Chinese Society of Matrix Biology (CSMB)
- Peking University
- Zhejiang University

The 11th Asian and Pan-Pacific Connective Tissue Societies Symposium (PPCTSS) & The 3rd National Conference of CSMB

Programs

Invited Speaker	Institute	Title
Reinhard Fässler	Max Planck Institute of Biochemistry	Genetic and biochemical analysis of focal adhesion proteins
Qingjun Meng	University of Manchester, UK	ECM-dependent circadian clocks in mammary epithelia: potential links to breast cancer
Xiaowei Li	Shanghai Jiaotong University	Whole-mount tissue clearing and imaging with single-cell resolution
Alain Colige	University of Liege, Belgium	The functions of ADAMTS2, 3 and 14 extend well beyond their aminoprocollagen peptidases activity
Francesco Ramirez	Icahn School of Medicine at Mount Sinai	Computational approaches to identify new drug treatments of aneurysmal disease
Jianfeng Chen	Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences	Integrin a4b7 switches its ligand specificity via distinct conformer-specific activation
Chuanyue Wu	Southern Medical University	Integrin signaling and human diseases
Jyrki Heino	Department of Biochemistry, University of Turku	Functional role of post-translational modifications in ECM proteins

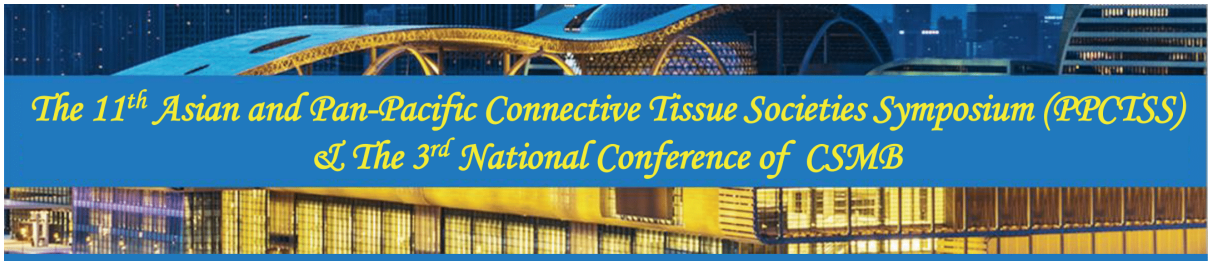


Programs

Invited Speaker	Institute	Title
Junlin Guan	University of Cincinnati College of Medicine	Focal adhesion kinase: the first quarter century and beyond
Jun Qin	Baylor College of Medicine	Molecular insight into the regulation of integrin activation and focal adhesion assembly
Xinhua Lin	Fudan University	Regulatory mechanisms of proteoglycans in endoderm patterning during development
James Whiteford	Queen Mary University Of London	A novel role for Syndecan-4 in neovascular diseases
Xuenong Zou	Institute of Orthopedics, the First Affiliated Hospital, Sun Yat-sen University	ECM and Intervertebral disc degeneration
Wei Kong	Peking University Health Science Center	*TBD*
John Whitelock	UNSW Sydney	The differential biological activities of the basement membrane heparan sulfate proteoglycan, perlecan in vascular, inflammatory and induced pluripotent stem cells
Lenna Bruckner	Dept. Dermatology, Medical Center - University of Freiburg	Translational skin matrix research: disease mechanisms and therapies

Programs

Invited Speaker	Institute	Title
Suneel S. Apte	American Chairman of Matrix Biology Society	Unexpected roles for metalloproteases in cell regulation
Hideto Watanabe	Japanese Chairman of Matrix Biology Society	Role of versican and its processing
Anthony J Day	Faculty of Biology, Medicine & Health, The University of Manchester	Glycosaminoglycan reorganization and crosslinking mechanisms in ovulation and inflammation
Jianglin Fan	University of Yamanashi	Functional roles of MMPs in the pathogenesis of atherosclerosis
Renato V. Iozzo	The editor of Matrix biology	Dual regulation of angiogenesis and autophagy by proteoglycans and its parts
Hongquan Zhang	Peking University Health Science Center	Kindlin-2 control fibrosis via multiple signaling pathway
Lin Wang	The Fourth Military Medical University	Sinusoidal endothelial niche and liver fibrosis
Wen Ning	Nankai University	Role of matricellular protein Fstl1 in lung development and fibrosis
Zhuowei Hu	Institute of Materia Medica, Peking Union Medical College	*TBD*



Programs

Invited Speaker	Institute	Title
Bo Huang	Peking Union Medical College	Killing the soft matrix for cancer treatment
Lydia Sorokin	University of Muenster, Germany	The Differential Role of the Endothelial Basement Membrane and the Gelatinases in Leukocyte Extravasation into the Brain
Liliana Schaefer	International Chairman of Matrix Biology Society	Advances in SLRP biology
Eok Soo Oh	Vice President, Office of Research; President, University-Industry Collaboration Foundation, EWHA WOMANS UNIVERSITY	Unique expression of syndecan-2 in proximal colon during acute inflammation
Chuanju Liu	New York University Medical Center	Interplay among ADAMTs and inflammatory cytokines in arthritis
Hongkui Deng	Peking University Health Science Center	Small molecules induced cell reprogramming
Bi-sen Ding	Weill Cornell Medicine	Vascular MMP14 regulates organ regeneration and fibrosis
Danny Chan	The University of Hong Kong	*Lgr5* and *Col22a1* mark differentiation of joint progenitor cells to juvenile articular chondrocytes

Programs

Invited Speaker	Institute	Title
Zhongjun Zhou	The University of Hong Kong	Isthmin is a novel inhibitor of nodal signaling
Jing Zhou	Peking University Health Science Center	Transduction of mechanical cues by discoidin domain receptor 1 and DNA methyltransferase 1
Yu Huang	Cardiovascular Research Center, The Chinese University of Hong Kong	From skeleton to cytoskeleton: osteocalcin transforms vascular fibroblasts to myofibroblasts via angiotensin II and Toll-like receptor 4
Congying Wu	Peking University Health Science Center	Gradient of matrix stiffness regulates cell migration and mitosis
Samuel I. Stupp	Northwestern University	Supramolecular Engineering of Bioactive Extracellular Matrices
Donald Gullberg	University of Bergen	*TBD*
Aifu Lin	Zhejiang University	*TBD*
Staffan Strömblad	Department of Biosciences and Nutrition, Clinical Molecular Biology Karolinska Institutet	Novel cell-matrix adhesion structures
Lynn Sakai	President of ASMB	*TBD*

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About Manchester

Manchester has grown from the first industrial city, the home of splitting of the atom and the first programmable computer, to be the central hub of the “Powerhouse of the North” of England – an influential, modern, knowledge-based city amongst the top European locations for science, healthcare, education, business and leisure.

The city of Manchester is situated centrally in mainland Britain, about two hours north of Birmingham and four hours outside of London. The region of Greater Manchester is home to some 2.5 million people, but as with the City of London, the City of Manchester has far fewer residents at just over 500,000. It is a city that has roots in ancient history but is now a bustling and unique metropolis.

Victorian architecture dominates the city, although the city's medieval roots can be seen around the Shambles as well as the 500 year old Chetham's School and Library buildings. Today, a new reputation is being built by bold and innovative local architects progressing one of the most ambitious regeneration programmes in Britain.

MUSIC, ARTS & CULTURE

From artistic masterpieces to the history of football, Manchester has an impressive range of museums and galleries, most of which are free. TheMuseum of Science and Industry brings innovation to life in the world’s oldest railway station, while the Imperial War Museum Northdemonstrates how war shapes people’s lives. Manchester is also home to the National Football Museum and the People’s History Museum all of which are free to enter.
The Whitworth , the gallery in the park, has recently been announced as the Art Fund's Museum of the Year 2015 and is home to an internationally important collection. Inspiring classic and contemporary art is also on show at the Manchester Art Gallery and you can find a plethora of smaller galleries all over the city. If you're interested in theatre you’ll find everything from opera to comedy to experimental at the Royal Exchange Theatre, HOME, Contact Theatre and The Lowry.
Chetham's Library, founded in 1653, is where Marx met Engels, has the oldest public library in the English-speaking world and is just a short walk from the main conference venue. Similarly the extraordinary and priceless John Rylands Library is close by on Deansgate, and hosts one of the finest collections of rare books, manuscripts and archives in the world.
Opportunities to experience live music in Manchester are as varied as they are exciting. The spectacular Bridgewater Hall is home to the Hallé Orchestra and welcomes musical talent from all over the world. Chamber music, folk, jazz, roots and drum & bass all have their place and major international acts perform regularly in the 20,000 seat Manchester Arena.

FOOD & DRINK

In a city as culturally diverse as Manchester, it's no surprise that the city has a great reputation for food and drink. You'll find cuisine from practically every nation and to suit any budget, from highclass dining to topnotch takeaways. We have the UK's second biggest Chinatown, where you'll find great Asian restaurants, and the neonlit 'Curry Mile' begins just a short walk from the city centre.

Restaurants, bars and clubs can be found in most parts of the city centre, from the bustling Deansgate Locks and The Printworks to the upmarketSpinningfields and the bohemian Northern Quarter.

SHOPPING

From stylish shopping malls and specialist market stalls to quirky boutiques and vintage treasure troves, the city offers a unique and diverse shopping experience. All of the UK's best known department stores, including Selfridges and Harvey Nichols are all within walking distance of the conference venue.

SPORT

There is no doubting Manchester's global reputation when it comes to sport. The city is home to world famous football teams and exceptional sporting facilities, so whether delegates want to watch or take part in sport, a fantastic visitor experience is guaranteed. Old Trafford, Lancashire County Cricket Club, the National Cycling Centre, home of British cycling, the Regional Tennis Centre and National Centre for Squash, all host international level sport for those who want to experience the electrifying atmosphere as a spectator.

For more information on sport in Manchester visit here.

Find out more about Manchester at www.visitmanchester.com/conference

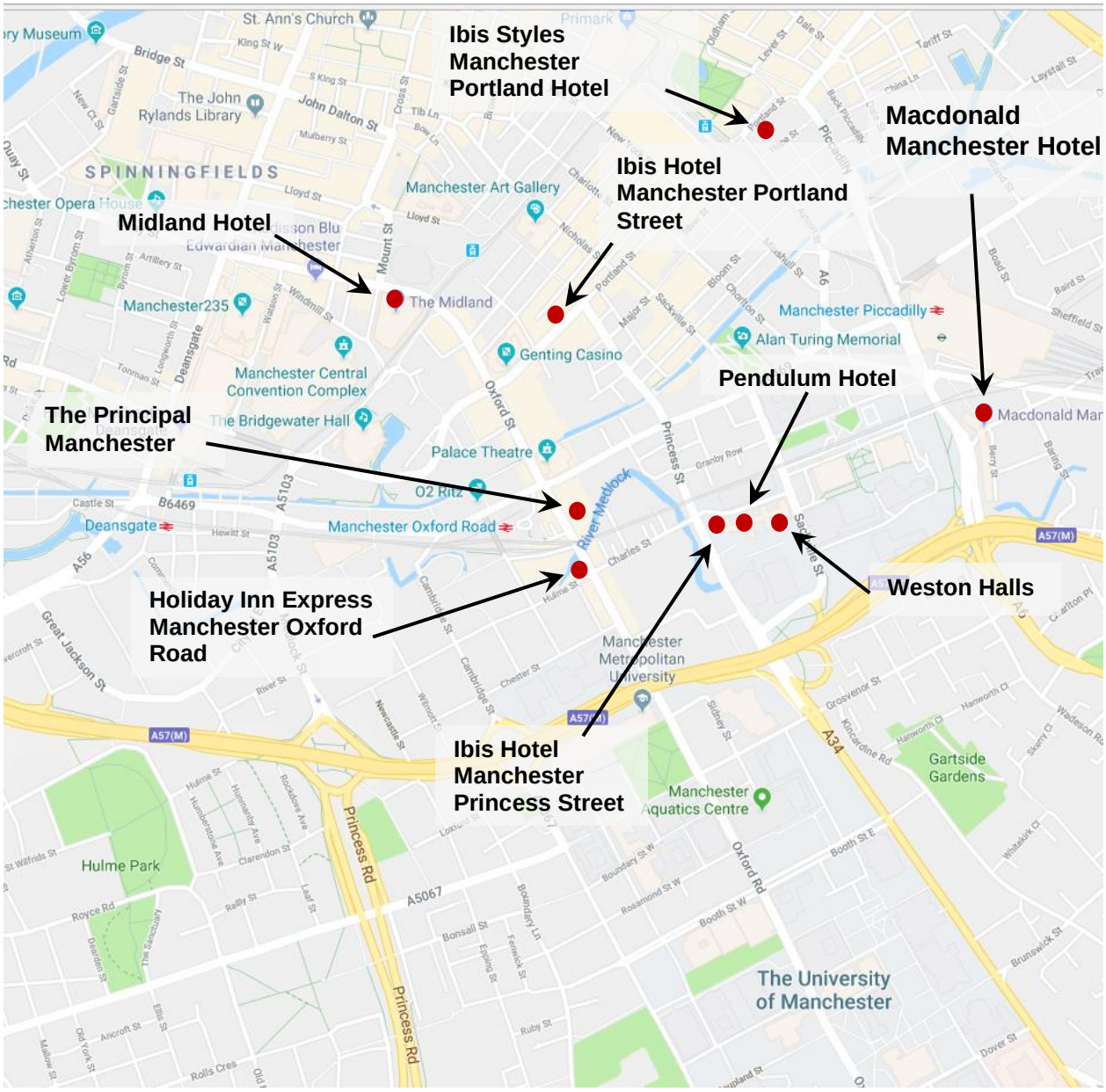
MBE Conference Location and City Map

Sir Matt Busby Way, Stretford, Manchester, M16 0RA

The University Of Manchester, Oxford Rd, Manchester M13 9PL

University Place, 176 Oxford Road, Manchester, M13 9PL

MBE Conference Accommodation



Midland Hotel, 16 Peter St, Manchester, M60 2DS

Ibis Styles, 3-5 Portland Street, Manchester, M1 6DP

Ibis Hotel, 96 Portland Street, Manchester, M1 4GX

Ibis Hotel, Charles Street, Princess Street, Manchester, M1 7DG

Macdonald Hotel, London Road, Manchester, M1 2PG

Pendulum Hotel, Sackville Street, Manchester, M1 3BB

Weston Halls, 78 Sackville Street, Manchester, M1 3BB

Holiday Inn Express, 2-4 Oxford Road, Manchester, M1 5QA

The Principal, Oxford Street, Manchester, M60 7HA

Attendance List

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Elena	Abraham	University of Köln	DE
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Patricia	Albanese	Université Paris-Est Créteil	FR
Julie	Albon	Cardiff University	GB
Judi	Allen	The University of Manchester	GB
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Anders	Aspberg	Lund University	SE
Paul	Atherton	The University of Manchester	GB
Man Ting	Au	Hong Kong Polytechnic University	HK
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Barbara	Bartolini	University of Insubria Varese- Italy	IT
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Peter	Bell	University of British Columbia	CA
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Roberta	Besio	University of Pavia	IT
Emma	Blain	Cardiff University	GB
Andy	Blanchard	GSK	GB
Ray	Boot-Handford	The University of Manchester	GB
George	Bou-Gharios	University of Liverpool	GB
Bent	Brachvogel	University of Köln	DE
Andrea	Brancaccio	CNR	GB
Dirk	Breitkreutz	German Cancer Research Center (retired)	DE
Stephane	Brezillon	CNRS UMR7369 Reims- France	FR
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Laura	Brightman	Geltor Inc	US
Peter	Bruckner	University of Muenster	DE
David	Buttle	University of Sheffield	GB
Adam	Byron	University of Edinburgh	GB
Huan	Cai	BMI- Copenhagen University	DK
Stuart	Cain	The University of Manchester	GB
Graciela	Calabrese	Universidad de Buenos Aires	AR
Ann	Canfield	University of Manchester	GB
Elena	Caravà	University of Insubria - Varese (ITALY)	IT
Giulia	Carini	University of Brescia	IT
Cheuk Wing Wilson	Chan	The University of Hong Kong	HK
Danny	Chan	The University of Hong Kong	HK

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Rita	Dreier	University of Muenster	DE
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Kati	Drushinin	University of Oulu	FI
Vic	Duance	Cardiff University	GB
Johanne	Dubail	Institut Imagine	FR
Laurent	Duca	University of Reims	FR
Michal	Dudek	The University of Manchester	GB
Laura	Dupont	University of Liege	BE
Douglas	Dyer	University of Glasgow	GB
Beate	Eckes	University of Cologne- Germany	DE
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Seda	Eminaga		GB
Hervé	Emonard	CNRS and University of Reims Champagne-Ardenne	FR
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marilena	formato	University of Sassari	IT
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Alan	Godwin	University of Manchester	GB
Frank	Gondelaud	Université Claude Bernard Lyon 1	FR
Ashleigh	Goodenough	University of Leeds	GB
Mike	Grant	The University of Manchester	GB
Tatiana	Gritsaenko	University of Nice	FR
Min	Guan	Chinese Academy of Sciences	GB
Farshid	Guilak	Washington University	US
Kevin	Hamill	University of Liverpool	GB
Uwe	Hansen	University Hospital Muenster	DE
Tim	Hardingham	The University of Manchester	GB
Ursula	Hartmann	Center Biochemistry	DE
Peleg	Hasson	Technion	IL
Christina	Hayward	The University of Manchester	GB
Jeremy	Herrera	The University of Manchester	GB
Tom	Hodgkinson	University of Glasgow	GB
Erhard	Hohenester	Imperial College	GB
Edward	Horton	University of Copenhagen	DK
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Wolfgang	Hübner	University Bielefeld	DE
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Martin	Humphries	The University of Manchester	GB
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Morten Mørk	Jensen	University of Southern Denmark	DK

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Konstantina	Karamanou	University of reims	GR
Craig	Keenan	University of Liverpool	GB
Oksana	Kehoe	Keele University at RJAH Orthopaedic Hospital	GB
Susan	Kimber	The University of Manchester	GB
András Attila	Kiss	University of Szeged	HU
Yvonne	Knieper	University of Muenster	DE
Anna	Köhler	Institute of Biochemistry	DE
Aljona	Kolmogorova	The University of Manchester	GB
Wei	Kong	PKUHSC	CN
Stefanie	Korntner	NUI Galway	IE
Nikki	Koudis	University of Manchester	GB
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Maike	Kümper	University of Köln	DE
Marie	Kveiborg	BRIC- University of Copenhagen	DK
Pui Yi	Kwan	the University of Hong Kong	HK
Laurence	Laegeai-Mallet	Institut Imagine	FR
Elizabeth	Laird	University of Liverpool	GB
Shireen	Lamande	Murdoch Children's Research Institute	AU
Alex	Langford-Smith	Manchester Metropolitan University	GB
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