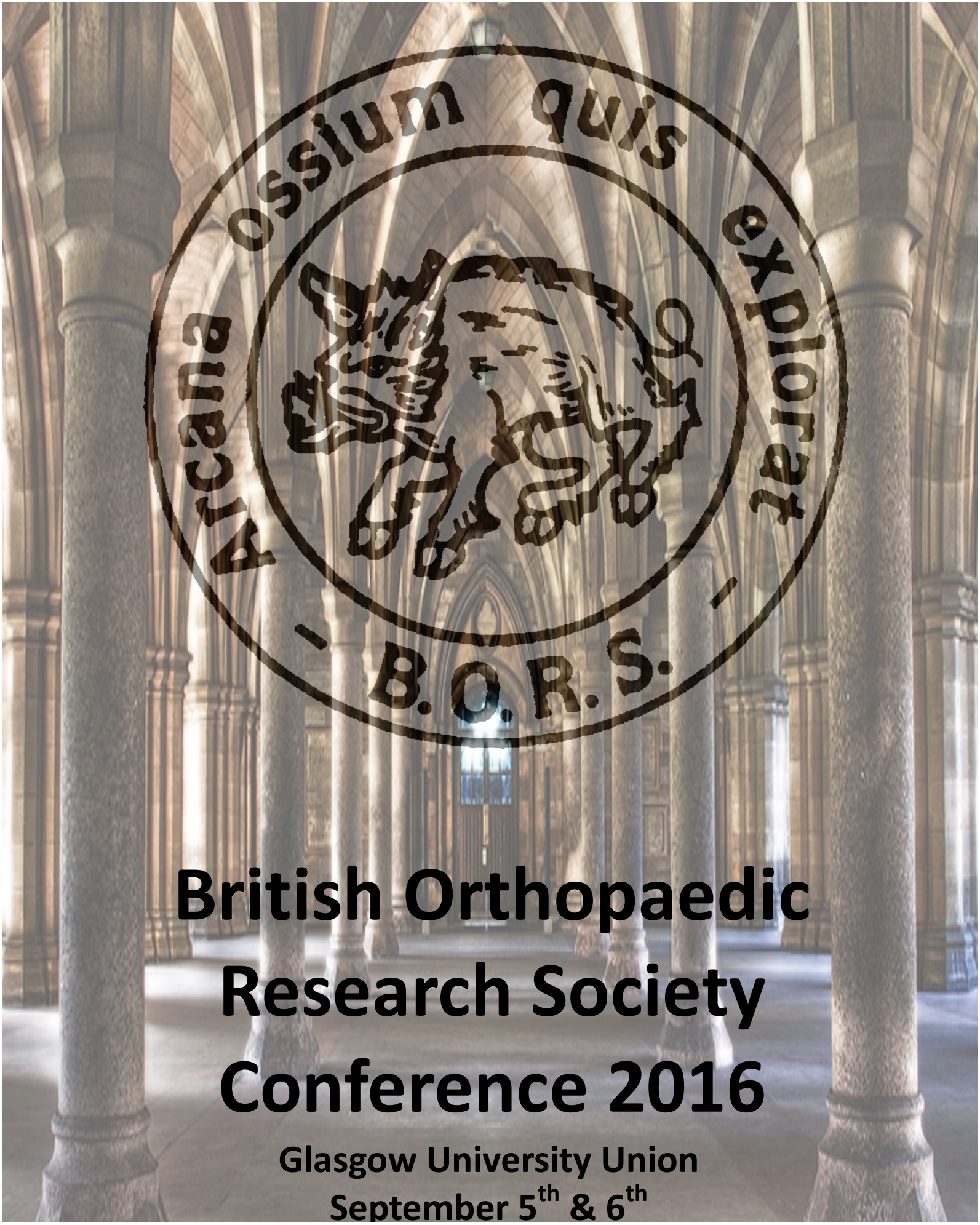


British Orthopaedic Research Society Conference 2016

Glasgow University Union

September 5th & 6th

Welcome to BORS 2016





Welcome from the Glasgow 2016 Chairs

We look forward to welcoming you to Glasgow for BORS 2016. We have put together an amazing line up of keynote speakers from clinical and academic backgrounds and this has helped us to put together an excellent programme of abstract talks. The programme is so full that we have two special break-out sessions on tendinopathy and on robotic surgery. The meeting is important to Glasgow as we are working hard to develop an international reputation in orthopaedic research. In 2009 we formed the Glasgow Orthopaedic Research Initiative (GLORI) to help trainees in the West of Scotland engage with fundamental and applied research. It is this GLORI group that have organised this meeting. The conference will take place in the west end of Glasgow - an area known for its cafes, pubs and restaurants as well as its architechture; we hope you will enjoy the meeting!

Prof MJ. Dalby, Mr RMD Meek, Mr PS Young, Dr PM. Tsimbouri, Prof M. Salmeron-Sanchez.
Glasgow 2016 BORS Chairs

With thanks to:
Prof A Ayoub, Dr S Coupard, Prof H Grant, Mr F Mahmood, Prof S Reid, Dr P Riches, Mr N Millar, Prof L Tanner.
Glasgow 2016 BORS Committee

Welcome from the President of BORS

Welcome to the annual meeting of the British Orthopaedic Research Society. It has been a long time since we had a BORS meeting in Scotland and it's really good to be in Glasgow. The committee at Glasgow has done a fantastic job in delivering a balanced program of biology, bioengineering and clinical investigations with representations from many UK centres specialising in various aspects of orthopaedic research. The program has a number of excellent keynote speakers and I believe that this is the first BORS meeting that has two breakout sessions. The meeting has something for everyone and the environment is very conducive for networking so please enjoy the meeting.

Prof G. Blunn
President BORS

Invited Speakers

PROF ANDRÉS J. GARCÍA

Invited International Keynote – *Rae S. and Frank H. Neely Chair and Regents' Professor Woodruff School of Mechanical Engineering, Georgia Institute of Technology, USA.*

Engineered Hydrogels for Cell Delivery and Bone Repair

Hydrogels, highly hydrated cross-linked polymer networks, have emerged as powerful synthetic analogs of extracellular matrices for basic cell studies as well as promising biomaterials for regenerative medicine applications. A critical advantage of these artificial matrices over natural networks is that bioactive functionalities, such as cell adhesive sequences and growth factors, can be incorporated in precise densities while the substrate mechanical properties are independently controlled. We have engineered poly(ethylene glycol) [PEG]-maleimide hydrogels for the controlled delivery of therapeutic proteins and human mesenchymal stem cells to heal critical-sized segmental bone defects in mice. Materials engineering to present integrin-specific, cell-adhesive peptides enhanced mesenchymal stem cell survival and engraftment and resulted in improved bone repair. Additionally, these materials can be engineered to reduce bacterial infections in the context of bone repair. These studies establish these engineered PEG hydrogels as promising biomaterial carriers for cell delivery, engraftment and enhanced tissue repair.

PROFESSOR JON CLASPER CBE

Invited Clinical Keynote - *Emeritus Defence Professor & Consultant Orthopaedic Surgeon, Visiting Professor in Bioengineering, Imperial College London, Clinical Lead, The Royal British Legion Centre for Blast Injury Studies.*

'Blasted Bones' - Skeletal failure after explosions!

As a Defence Professor in Trauma & Orthopaedics he is responsible for the orthopaedic research focus of the British military, and a founding member of the Imperial Blast group. Professor Clasper will talk to us about his experiences in dealing with blast trauma and its effect on the skeleton.

PROFESSOR LIAM GROVER

Invited Academic keynote - *Professor in Biomaterials Science, Director of Research, University of Birmingham.*

Manipulating ossification through chemistry

Although inorganic materials such as hydroxyapatite and calcium sulphate are widely used for the reconstruction of hard tissue defects or for coating metallic prosthetics, few recognise the importance and therefore potential of a range of more complex inorganic materials to control the process of ossification. This talk will discuss how making subtle changes to the chain length of phosphate ions can generate materials that can bond bone, trigger extensive bone formation or even demineralise hard tissue. It will describe how we are utilising this complexity to aid in the repair of non-union and the regeneration of larger bone defects. Understanding the complexities of the mineralisation process has also enabled us to generate 3D *in vitro* models of bone that can maintain osteocyte viability for in excess of one year. These models have been used as a platform to screen novel treatments for the dispersion on heterotopic ossification.

MR MARK BLYTH

Invited Clinical keynote – *Consultant Orthopaedic Surgeon, Glasgow Royal Infirmary.*

Robots in knee osteoarthritis: A compartmentalised solution?

Mr Blyth will present in and chair the STRYKER sponsored session on precision orthopaedics. This session will focus on the drive for precision and accuracy in orthopaedics, with a keynote talk on the evolving role of robotic assistance in orthopaedic surgery, particularly unicompartmental and bi-unicompartmental knee arthroplasty. This session will also feature presentations on implant design, through modern manufacturing processes to precision surgical techniques.

DR PAUL GENEVER

Invited Academic keynote – *University of York.*

Bone regeneration: Making the most of MSCs

Bone marrow mesenchymal stromal cells (MSCs) are heterogeneous cell populations that are likely to contain rare stem cell subsets as well as additional, functionally-important non-stem cell fractions. We have generated several immortalised, clonal human MSC lines to study functional heterogeneity and we have identified significant variation in potency and immunoregulatory profiles. The MSC lines can be grown as 3D spheroids and genetically modified, for example using CRISPR/Cas9, to introduce insertion/deletion mutations to model and track skeletogenic programmes and disease onset/progression. This talk will cover some of our work in these areas and explain how disentangling the biological versatility of MSCs can deepen our understanding of MSC function and advance new therapeutic development particularly for bone and joint disorders.

PROFESSOR JOHN KENWRIGHT

Recipient: Presidential Prize for Outstanding Contribution to the Field of Orthopaedic Research

The seven sins of research: resisting temptation

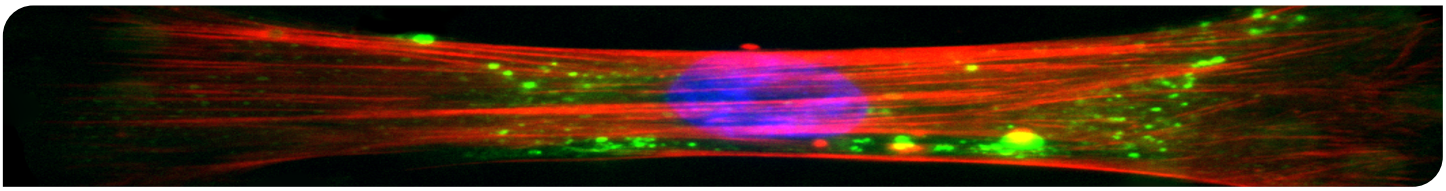
Professor Kenwright is the recipient of the BORS presidential prize for outstanding contribution to orthopaedic research. He completed his medical training at St Johns's college, Oxford, and the University College Hospital, London before higher degree at the Karolinska Institute. He was the 5th Nuffield Professor of orthopaedic surgery from 1992 – 2001. In recognition of his enormous contribution to research within the field of orthopaedics he is to be awarded the BORS Presidential Prize.

PROFESSOR STUART REID

Invited Public Lecture – *University of West of Scotland*

Gravity waves, stem cells and bone regeneration

Stuart is part of the international team behind the first detection of gravitational waves in 2015, associated with two black holes colliding, and heralded by many as the scientific breakthrough of the century. He has been centrally involved in developing interferometry-based technology, which has been used alongside cell biologists to develop nanokicking, a technology pioneered in Glasgow for turning stem cells into bone.



Programme in Brief

MONDAY 5TH SEPTEMBER

08:00 – 9:00 CONFERENCE REGISTRATION AND REFRESHMENTS
09:00 – 9:05 WELCOME TO BORS 2016
09:05 – 10:30 STEM CELL SESSION: DEBATES CHAMBER
- 9:05 – 9:35 KEYNOTE: PROFESSOR PAUL GENEVER
- 9:35 – 10:30 FREE PAPER SESSION

10:30 – 11:00 COFFEE & REFRESHMENTS IN THE READING ROOM. POSTERS SESSION VIEWING.

11:00 – 13:00 BIOMATERIALS BONE REMODELLING: DEBATES CHAMBER
- 11:00 – 12:00 KEYNOTE: PROFESSOR ANDRÉS GARCÍA
- 12:00 – 13:00 FREE PAPER SESSION: BIOMATERIALS

11:00 – 13:00 MOLECULAR TENDINOPATHY BREAKOUT SESSION: BRIDIE LIBRARY
13:00 – 14:00 LUNCH SERVED IN THE DINING ROOM (BORS AGM IN THE DEBATES CHAMBER)

14:00 – 15:30 PRECISION ORTHOPAEDICS AND ADVANCED MANUFACTURING: DEBATES CHAMBER
- 14:00 – 14:30 KEYNOTE: MR MARK BLYTH
- 14:30 – 15:00 FREE PAPER SESSION & STRYKER SPONSORED TALKS

15:30 – 16:00 COFFEE AND REFRESHMENTS SERVED IN THE READING ROOM. POSTER VIEWING.

15:30 – 17:00 BREAKOUT SESSION WITH STRYKER EXPERTS: BRIDIE LIBRARY
16:00 – 17:00 INFECTION IN ORTHOPAEDICS FREE PAPER SESSION: DEBATES CHAMBER
17:00 – 17:30 PRESIDENTIAL PRIZE LECTURE: DEBATES CHAMBER
17:30 CLOSE, THANKS AND DRINKS AVAILABLE IN DINING HALL UNTIL DINNER
19:00 ARRIVAL FOR DRINKS RECEPTION AND DINNER
21:30 – 00:00 AFTER DINNER SPEECHES & CEILIDH

TUESDAY 6TH SEPTEMBER

08:30 – 9:30 BIOMECHANICS FREE PAPER SESSION: DEBATES CHAMBER
09:30 – 10:30 ACADEMIC OSTEOARTHRITIS FREE PAPER SESSION: DEBATES CHAMBER
10:30 – 11:00 COFFEE & REFRESHMENTS IN THE READING ROOM. POSTERS SESSION VIEWING.

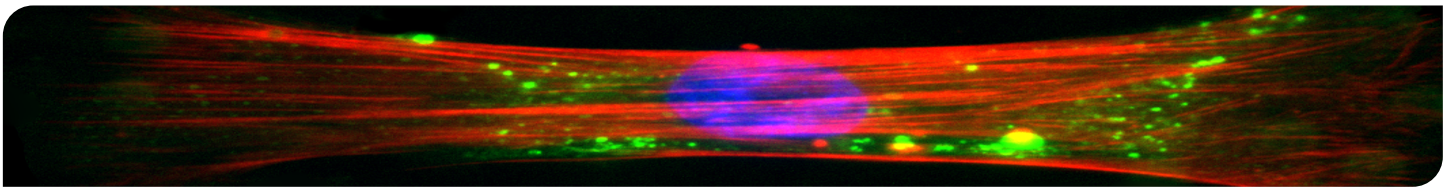
11:00 – 13:00 BLASTED BONES AND WORN OUT JOINTS
- 11:00 – 11:50 KEYNOTE: PROFESSOR JON CLASPER
- 11:50 – 13:00 FREE PAPER SESSION: CLINICAL OSTEOARTHRITIS

13:00 – 14:00 LUNCH SERVED IN THE DINING ROOM

14:00 – 15:30 BONE MINERALISATION & OSTEOPOROSIS
- 14:00 – 14:30 KEYNOTE: PROFESSOR LIAM GROVER
- 14:30 – 15:30 FREE PAPER SESSION MINERALISATION & OSTEOPOROSIS

15:30 – 16:00 COFFEE AND REFRESHMENTS SERVED IN THE READING ROOM. POSTER VIEWING.

16:00 – 16:30 INVITED PUBLIC LECTURE: PROFESSOR STUART REID
16:35 – 17:00 PRESENTATION PRIZES, CLOSE OF CONFERENCE



Monday 5th September

Morning Session: Debates Chamber

8:00 – 9:00 CONFERENCE REGISTRATION AND REFRESHMENTS

9:00 – 9:05 WELCOME TO BORS 2016: PROF G BLUNN, PROF MJ DALBY AND MR RMD MEEK.

9:05 – 10:30 STEM CELL SESSION

CHAIRING: DR R. WALLACE & MR R. SILVERWOOD

9:05 – 9:35 KEYNOTE: BONE REGENERATION: MAKING THE MOST OF MSC'S
PROFESSOR PAUL GENEVER
Reader, Department of Biology, University of York.

9:35 – 10:30 FREE PAPER SESSION

MODULATING OSTEOGENESIS IN HUMAN MSCS BY GOLD NANOPARTICLE DELIVERY OF MIR 31A ANTAGOMIRS
A Vatsa, MA McCully, Joao Conde, Pedro Baptista, Helen Wheadon, M Dalby , C Berry.
Centre for Cell Engineering, Institute of Molecular, Cell & Systems Biology, CMVLS, University of Glasgow, Glasgow.

PTH 1-34 EFFECTS THE DIFFRENTIATION OF BONE MARROW STEM CELL DERIVED ADIPOCYTES
L. Clouard, A. Sanghani-Kerai, M. Coathup, T. Briggs, G. Blunn.
Institute of Musculoskeletal Sciences, University College London, RNOH, Stanmore HA7 4LP.

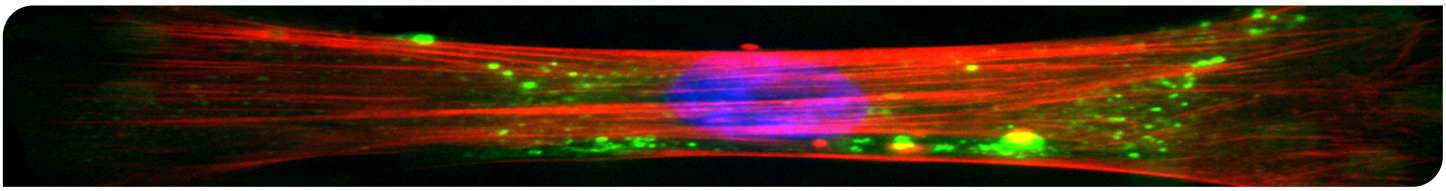
THE MAGNETIC LABELLING OF STEM CELLS TO ENGINEER THE BONE MARROW NICHE *IN VITRO*
N. Lewis, E. Lewis, M. Dalby & C. C. Berry.
Centre for Cell Engineering, Joseph Black Building, University Avenue, University of Glasgow, Glasgow.

DIFFERENCES IN MORPHOLOGY, PROLIFERATION AND IMMUNE PROFILE AMONG SINGLE-CELL CLONED STEM CELLS FROM THE SAME MESENCHYMAL STEM CELL ORIGIN
Y. Cheng, S. Sorousheh, M. Coathup, G. Blunn.
John Scales Centre for Biomedical Engineering, Royal National Orthopaedic Hospital, Stanmore, UK.

MATERIAL-DRIVEN FIBRONECTIN NANONETWORKS AS EFFICIENT BMP2 MICROENVIRONMENTS FOR BONE REPAIR
C. Gonzalez-Garcia, V. Llopis-Hernandez, D. Shields, M. Cantini, A. Alba, A.J. Garcia, M. Dalby, M. Salmeron-Sanchez
Biomedical Engineering Research Division, School of Engineering, University of Glasgow, UK.

USING SURFACE CHEMISTRY TO REGULATE BEHAVIOUR OF MESENCHYMAL STEM CELLS
P Sweeten, R Gurden, L-A Turner, E Ross, M. Salmeron-Sanchez, J Mountford, M.J. Dalby.
Centre for Cell Engineering, Institute of Molecular, Cell, and Systems Biology, University of Glasgow, UK.

10:30 – 11:00 COFFEE & REFRESHMENTS IN THE READING ROOM. POSTERS SESSION VIEWING.



Monday 5th September

Mid - Morning Session: Debates Chamber

11:00 – 13:00 BIOMATERIALS: BONE REMODELLING

CHAIRING: PROF M. WILKINSON & DR M. CANTINI

11:00 – 12:00 KEYNOTE: ENGINEERED HYDROGELS FOR CELL DELIVERY AND BONE REPAIR
PROFESSOR ANDRÉS GARCÍA
Rae S. and Frank H. Neely Chair and Regents' Professor Woodruff School of Mechanical Engineering. Georgia Institute of Technology, USA.

12:00 – 13:00 FREE PAPER SESSION: BIOMATERIALS

PHOSPHONATE-TETHERED LYSOPHOSPHATIDIC ACID-FUNCTIONALISED TITANIUM: A NOVEL SURFACE FINISH FOR CEMENTLESS ORTHOPAEDIC IMPLANTS.
W Nishio Ayre, T Scott, K Hallam, AW Blom, SP Denyer, H Bone, JP Mansell
School of Dentistry, Cardiff University, Cardiff CF14 4XY, UK.

PRECISION ENGINEERED STRONTIUM ELUTING NANOTOPOGRAPHICAL SURFACES TO CONTROL BONE FORMATION
P.S. Young, A.I.M. Greer, P.M. Tsimbouri, R.M.D Meek, N. Gadegaard, M.J. Dalby
Centre for Cell Engineering, University of Glasgow, Glasgow.

DEVELOPMENT OF AN ADAPTIVE BONE REMODELING MODEL DRIVEN BY MECHANICAL AND BIOLOGICAL STIMULI FOR IMPLANT ANALYSIS
V. S. Cheong, M. J. Coathup, A. Mumith, P. Fromme, G. W. Blunn
Institute of Orthopaedics and Musculoskeletal Science, University College London, Middlesex HA7 4LP.

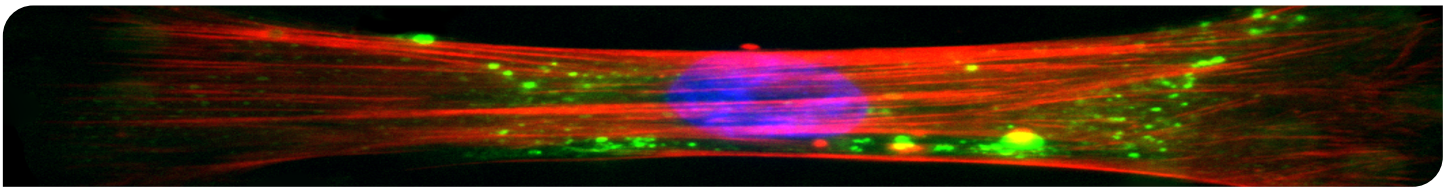
3D PRINTED PLA SCAFFOLDS FOR BONE TISSUE REGENERATION: EFFECT OF SCAFFOLD STRUCTURE ON ATTACHMENT AND GROWTH OF HUMAN DENTAL PULP STROMAL CELLS (HDPSCS)
R.F. Albannaa, J. Kirkham, J. Burke, C. Liu and X. Yang
Biomaterials and Tissue Engineering Group, Department of Oral Biology, School of Dentistry, University of Leeds.

ENGINEERING OSTEOGENIC COATINGS ON PEEK
V. Llopis-Hernandez, D. Sharp, A. Alba-Perez, M. J. Dalby, M. Salmeron-Sanchez
Division of Biomedical Engineering, School of Engineering, University of Glasgow.

GROWTH BY STRETCH: AN INTERDISCIPLINARY APPROACH TO IMPROVE CURRENT PRACTICE
R.Unadkat, M.Riehle, R.Burchmore, A.Hart.
Centre of Cell Engineering, University of Glasgow, G12 8QQ.

13:00 – 14:00 LUNCH SERVED IN THE DINING ROOM

13:00 – 14:00 BORS AGM IN THE DEBATES CHAMBER



Monday 5th September

Mid - Morning Breakout Session: Bridie Library

11:00 – 13:00 MOLECULAR TENDINOPATHY: TOWARD TRANSLATION

CHAIRING: MR N.L. MILLAR

11:05 – 11:30 INVITED LECTURE: INFLAMMATION, ACTIVATION AND RESOLUTION IN TENDINOPATHY
DR STEPHANIE G DAKIN, UNIVERSITY OF OXFORD

11:30 – 11:50 FREE PAPER SESSION: TENDON

PROTEINASE-ACTIVATED RECEPTOR 2: POTENTIAL ROLE IN OSTEOARTHRITIC ENTHESEAL PATHOLOGY
A. Ortiz, L. Dunning, C. Huesa, W.R. Ferrell, I.B. McInnes, J.C. Lockhart, N.L. Millar, C.S. Goodyear, A. Crilly.
University of WOS and Institute of Infection, Immunity and Inflammation, MVLS, University of Glasgow, UK

DEVELOPING A NOVEL 3-DIMENSIONAL (3D) CO-CULTURE SYSTEM TO GENERATE A TENDON-BONE TISSUE INTERFACE: EVALUATION OF HYDROGELS
H. Alsaykhan and J.Z. Paxton
Hugh Robson Building, Edinburgh Medical School: Biomedical Sciences, University of Edinburgh, EH8 9XD.

11:50 – 12:15 INVITED LECTURE: PROTEOMIC AND TRANSCRIPTOMIC SIGNATURES IN TENDON DISEASE
DR MANDY PEFFERS, UNIVERSITY OF LIVERPOOL

12:15 – 12:35 FREE PAPER SESSION: TENDON

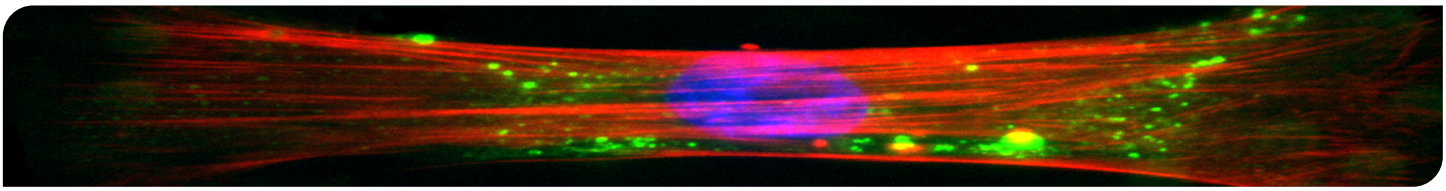
THE EFFECTS OF TERMINAL STERILISATION USING VARYING IRRADIATION DOSAGES ON THE BIOMECHANICAL PROPERTIES OF THE ACELLUAR PORCINE FLEXOR TENDON.
A.Herbert, J.H. Edwards, E. Ingham, J. Fisher.
Institute of Medical and Biological Engineering, University of Leeds. UK.

S100 PROTEINS: DANGER SIGNALS IN TENDINOPATHY
L.A.N. Crowe, M. Akbar, S.M. Kitson, J.H. Reilly, S.C. Kerr, I.B. McInnes, D.S. Gilchrist, N.L. Millar
Institute of Infection, Immunity and Inflammation, MVLS, University of Glasgow, UK.

12:35 – 13:00 INVITED LECTURE: NEURAL PATHWAYS IN TENDINOPATHY
MR BENJAMIN JF DEAN, UNIVERSITY OF OXFORD.

13:00 – 14:00 LUNCH SERVED IN THE DINING ROOM

13:00 – 14:00 BORS AGM IN THE DEBATES CHAMBER



Monday 5th September

Afternoon Session: Debates Chamber

14:00 – 15:30 PRECISION ORTHOPAEDICS AND ADVANCED MANUFACTURING

CHAIRING: MR M. BLYTH & DR D. HANSOM

14:00 – 14:30 KEYNOTE: ROBOTS IN KNEE OA: A COMPARTMENTALISED SOLUTION?
MR MARK BLYTH
Orthopaedic Consultant, Glasgow Royal Infirmary, Glasgow, UK.

14:30 – 15:00 FREE PAPER SESSION PRECISION ORTHOPAEDICS

CREATING THE NEXT GENERATION OF ORTHOPAEDIC FIXATION SCREWS – A NOVEL USE OF 3-D PRINTING
L. J. Leslie, G. Heaven, J. G. Swadener, S. Junaid, K. Theivendran, S. C. Deshmukh
School of Engineering and Applied Science, Aston University, Birmingham, B4 7ET.

DEVELOPMENT OF AN AUGMENTED REALITY-GUIDED COMPUTER ASSISTED ORTHOPAEDIC SURGERY SYSTEM
N. L. Smith, V. Stankovic, and P. E. Riches
Department of Biomedical Engineering, University of Strathclyde, Wolfson Centre, Glasgow, G4 0NW.

3D PRINTING OF ORTHOPAEDIC CLINICAL IMAGES TO AID COMPLEX SURGICAL PLANNING: A PROOF OF CONCEPT STUDY
MTA Griffin, J Annan, DF Hamilton, AHRW Simpson.
Department of Orthopaedics and Trauma, University of Edinburgh, 49 Little France Crescent, EH16 4SB.

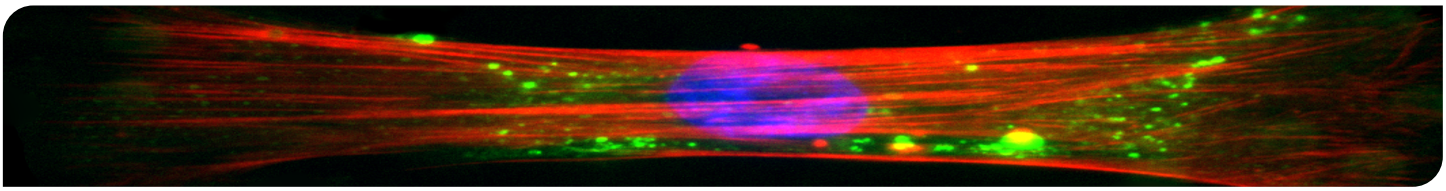
15:00 – 15:15 STRYKER MODELLING AND ANALYTICS SYSTEM: FUTURE IMPLANT DESIGN
ANDREAS PETERSIK, R&D VIRTUAL ENGINEERING

15:15 – 15:30 STRYKERS ADDITIVE MANUFACTURING: MAKING THE COMPLEX POSSIBLE
GEAROID WALSH, SENIOR ENGINEER, AO ADDITIVE.

15:30 – 16:00 COFFEE AND REFRESHMENTS SERVED IN THE READING ROOM. POSTER VIEWING.

Late Afternoon Breakout Session: Bridie Library

15:30 – 17:00 BREAKOUT SESSION WITH STRYKER EXPERTS
ANDREAS PETERSIK & GEAROID WALSH WILL BE AVAILABLE WITH OTHER STYRKER STAFF AND SAWBONES WORKSHOPS IN THE BRIDIE LIBRARY TO ANSWER ANY TECHNICAL QUESTIONS FOLLOWING THE PRECISION ENGINEERING SESSION.



Monday 5th September

Late - Afternoon Session: Debates Chamber

16:00 – 17:00 INFECTION IN ORTHOPAEDICS

CHAIRING: PROF G. RAMAGE & MR I. SMITH

16:00 – 17:00 FREE PAPER SESSION

A MURINE MODEL OF SEPTIC ARTHRITIS DEMONSTRATES THAT INFECTION WITH AN ALPHA TOXIN PRODUCING *STRAIN OF S. AUREUS* LEADS TO SIGNIFICANTLY ELEVATED LEVELS OF CHONDROCYTE DEATH WITHIN 48 HOURS
R. Clement, A. Hall, S. Howie, H. Simpson.
Department of Trauma and Orthopaedics, Morriston Hospital, Heol Maes Eglwys, Morriston, Swansea. SA6 6NL.

NANOCARRIER DELIVERY OF BIOACTIVE MATRICES AND ANTIMICROBIALS FOR HARD TISSUE REPAIR
G. E. Melling, S. J. Avery, S. L. Evans, R. J. Waddington, and A. J. Sloan
Mineralised Tissue Group, Oral and Biomedical Sciences, Cardiff University, Cardiff.

DEVELOPMENT OF 405 NM HINS-LIGHT TECHNOLOGY FOR DECONTAMINATION APPLICATIONS IN ARTHROPLASTY
P. Ramakrishnan, M. Maclean, S. J. MacGregor, J.G. Anderson, M. H. Grant.
University of Strathclyde, Department of Biomedical Engineering, Glasgow, Scotland G4 0NW, United Kingdom.

THE EFFECTS OF NANOPATTERN SURFACE TECHNOLOGY ON ORTHOPAEDIC JOINT REPLACEMENT INFECTION.
D.Hansom, G.Ramage, K.Burgess, N.Gadengaard, N.Millar, J.Clarke.
School of Medicine, College of Medical, Veterinary & Life Sciences, Dental Hospital, University of Glasgow.

OSTEOGENIC AND BACTERICIDAL SURFACES FROM HYDROTHERMAL TITANIA NANOWIRES ON TITANIUM SUBSTRATES
P.M. Tsimbouri, N. Holloway, L. Fisher, T. Sjostrom. A.H. Nobbs, R.M.D Meek, B. Su, M.J. Dalby.
Centre for Cell Engineering, University of Glasgow, Glasgow, Scotland, UK.

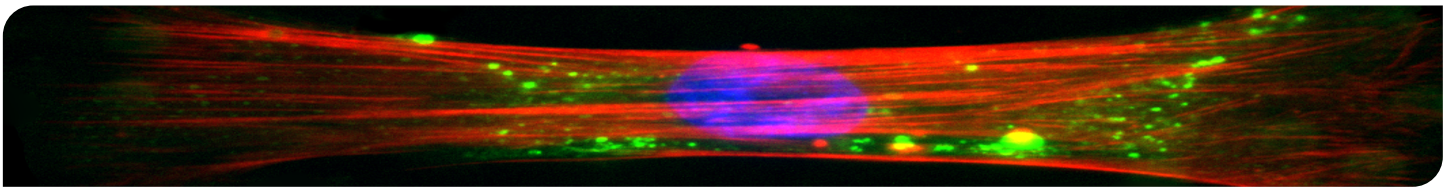
HOW DOES THE LEVEL OF BONE MINERALISATION AFFECT THE DEPTHS PROBED BY SPATIALLY OFFSET RAMAN SPECTROSCOPY?
K. Sowoidnich, J. H. Churchwell, K. Buckley, J. G. Kerns, A. E. Goodship, A. W. Parker, P. Matousek.
Central Laser Facility, STFC Rutherford Appleton Laboratory, Harwell Campus OX11 0FA, UK.

17:00 – 17:30 PRESIDENTIAL PRIZE LECTURE: PROF J. KENWRIGHT
CHAIRING: PROF G. BLUNN

17:30 CLOSE, THANKS AND DRINKS AVAILABLE IN DINING HALL UNTIL DINNER

19:00 ARRIVAL FOR DRINKS RECEPTION AND DINNER

21:30 AFTER DINNER SPEECHES & CEILIDH



Tuesday 6th September

Morning Session: Debates Chamber

8:30 – 9:30 BIOMECHANICS SESSION

CHAIRING: PROF E. TANNER & MR F. MAHMOOD

8:30 – 9:30 FREE PAPER SESSION

ROTATIONAL ALIGNMENT OF THE DISTAL FEMUR IN TOTAL KNEE ARTHROPLASTY: AN MRI ANALYSIS
M. Halai, B.Jamal, P. Robinson, M.Qureshi, J.Kimpton, B.Syme, J.McMillan, G.Holt.
Crosshouse Hospital, Glasgow, United Kingdom.

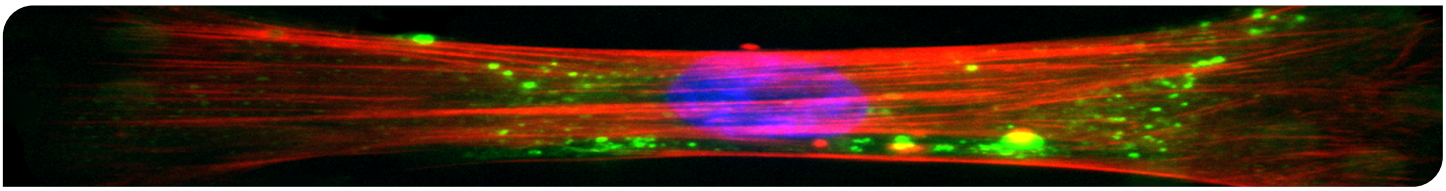
STRAIN FIELD MEASUREMENTS APPLIED TO ARTICULAR CARTILAGE USING DIGITAL IMAGE CORRELATION
K.B. Czerbak, S.E. Clift, S. Gheduzzi.
Centre for Orthopaedic Biomechanics, University of Bath, Claverton Down Rd, Bath, North East Somerset BA2 7AY

A NEW LANDMARK FOR MEASURING TIBIAL ROTATION AFTER TOTAL KNEE REPLACEMENT
N. Holloway, A. Deakin, F. Picard
Department of Orthopaedics, Golden Jubilee National Hospital, Dunbartonshire, Glasgow, G81 4DY.

IDENTIFICATION OF MOVEMENT STRATEGIES DURING THE SIT-TO-WALK MOVEMENT IN PATIENTS WITH KNEE OSTEOARTHRITIS.
D. S. Komaris, C. Govind, P. Riches, A. Murphy, A. Ewen, F. Picard, J. Clarke
Department of Biomedical Engineering, University of Strathclyde, 106 Rottenrow East, Glasgow, G4 0NW.

THE EFFECT OF K TAPE ON VASTUS MEDIALIS OBLIQUE AND RECTUS FEMORIS MUSCLE ACTIVITY AND CRTICAL KNEE FLEXION ANGLE AT WHICH DYNAMIC VALGUS OCCURS DURING A SINGLE- LEG SQUAT.
L Fitzgerald V Sparkes.
Arthritis Research UK Biomechanics and Bioengineering Centre, Cardiff University, Cardiff , UK, CF 24 0AB.

MEASURING CORTICAL BONE STIFFNESS USING MICRO-INDENTATION
O.Boughton, S.Zhao, M.Arnold, S.Ma, J.P.Cobb, F.Giuliani, U.Hansen, R.L.Abel.
The Musculoskeletal Lab, 7L16, Lab Block, Charing Cross Campus, Imperial College London, W6 8RP, UK.



Tuesday 6th September

Morning Session: Debates Chamber

9:30 – 10:30 ACADEMIC OSTEOARTHRITIS SESSION

CHAIRING: PROF G. BLUNN & DR C. GOODYEAR

9:30 – 10:30 FREE PAPER SESSION

PATIENT MUSCLE SATELLITE CELL CONTENT IS A POTENTIAL BIOMARKER FOR PHYSCIAL RECOVERY AND CLINICAL OUTCOME FOLLOWING TOTAL KNEE ARTHROPLASTY

DF Hamilton, P Gaston, AHRW Simpson.
Department of Orthopaedics, University of Edinburgh, 49 Little France Crescent, Edinburgh, EH16 4SB.

TRIM32 DEFICIENCY IS ASSOCIATED WITH INCREASED CARTILAGE DEGRADATION AND ALTERED CHONDROCYTE PHENOTYPE IN HUMAN AND MURINE ARTICULAR TISSUE *EX VIVO*

S.Roberts, D.Salter, S.Ralston.
Bone Research Group, IGMM, University of Edinburgh.

THE EFFECTS OF CHRONIC COBALT AND CHROMIUM EXPOSURE AFTER METAL-ON-METAL HIP RESURFACING ON DNA METHYLATION: AN EPIGENOME-WIDE ASSOCIATION STUDY

J Steinberg, KM Shah, A Gartland, E. Zeggini, JM Wilkinson.
Wellcome Trust Sanger Institute, Cambridge, UK.

COMPARISON OF THE CYTOTOXTIC EFFECTS OF CLINICALLY-RELEVANT COBALT-CHROMIUM WEAR PARTICLES AND COMMERCIAL COMPOSITE CERAMIC PARTICLES

I M. Asif, S. Williams, J. Fisher, M. Al-Hajjar, J. Anderson, J L. Tipper
Institute of Medical & Biological Engineering, University of Leeds, Leeds, LS2 9JT, UK.

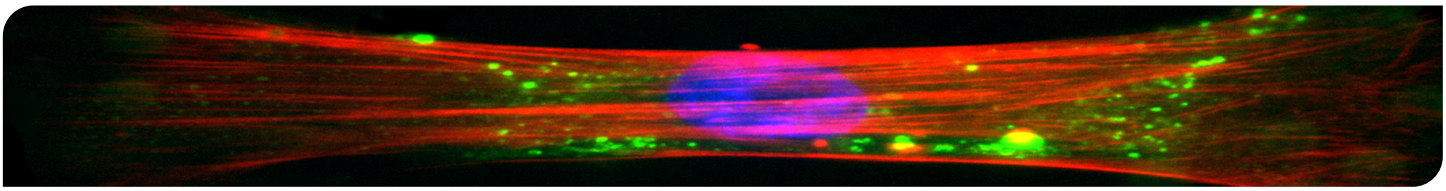
EFFECT OF CIRCULATING METAL IONS ON SURVIVAL AND FUNCTION OF OSTEOCLASTS FROM PATIENTS FOLLOWING HIP RESURFACING

KM Shah, PN Sudsok, D Morrell, A Gartland, JM Wilkinson.
Department of Oncology and Metabolism, Mellanby Centre for Bone Research, University of Sheffield, Sheffield.

OSTEOCLAST PREVALENCE WITHIN BONE MARROW LESIONS AND SYNOVITIS SEVERITY AND THEIR ASSOCIATION WITH NEUROPATHIC PAIN AND CENTRAL SENSITIZATION IN KNEE OSTEOARTHRITIS

T.Kurien, R.Kerslake, BE. Scammell and RG.Pearson
Arthritis Research UK Pain Centre, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH.

10:30 – 11:00 COFFEE & REFRESHMENTS IN THE READING ROOM. POSTERS AVAILABLE TO VIEW.



Tuesday 6th September

Mid - Morning Session: Debates Chamber

11:00 – 13:00 BLASTED BONES AND WORN OUT JOINTS

CHAIRING: MR R.M.D. MEEK & MR P.S. YOUNG

11:00 – 11:50 KEYNOTE: BLASTED BONES! SKELETAL FAILURE AFTER EXPLOSIONS
PROFESSOR JON CLASPER
Professor in Bioengineering, Imperial College London, Clinical Lead, The Royal British Legion Centre for Blast Injury Studies.

11:50 – 13:00 FREE PAPER SESSION: CLINICAL OSTEOARTHRITIS

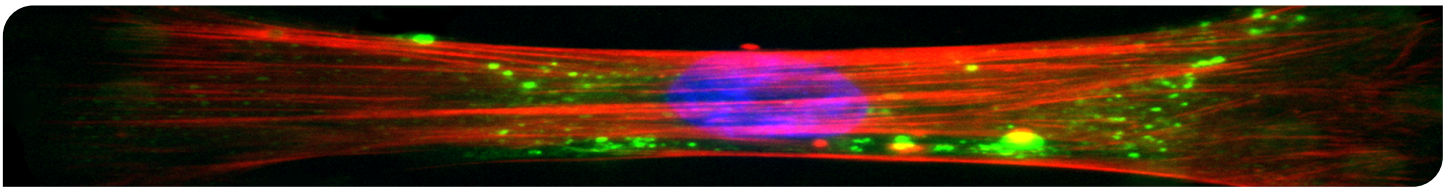
HOW DOES IMPLANT TYPE, HEAD SIZE AND AVN LESION SIZE AFFECT THE LIKELIHOOD OF FEMORAL HEAD COLLAPSE FOLLOWING HIP FIXATION?
A. R. MacLeod, M. Whitehouse, H. S. Gill, E. C. Pegg
Centre for Orthopaedic Biomechanics, University of Bath, Bath, UK

PREDICTING CHRONIC POST-OPERATIVE PAIN AFTER TKR BY PREOPERATIVE ASSESSMENT OF CENTRAL SENSITIZATION USING FUNCTIONAL BRAIN MRI AND SEMI-QUANTITATIVE MRI SCORING OF THE KNEE. A PRELIMINARY CASE-CONTROL STUDY.
T.Kurien, D. Reckziegel, WJ. Cottam, KK. Petersen, L.Ardent-Nielsen, T.Graven-Nielsen, RG.Pearson, DP. Auer, BE. Scammell
Arthritis Research UK Pain Centre, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH.

PATIENT FUNCTION FOLLOWING ASEPTIC REVISION TOTAL KNEE ARTHROPLASTY WITH SEMI-CONSTRAINED PROSTHESES IS THE SAME AS FOLLOWING PRIMARY KNEE ARTHROPLASTY
DF Hamilton, P Simpson, JT Patton, CR Howie, R Burnett
Department of Orthopaedics, University of Edinburgh, 49 Little France Crescent, Edinburgh, EH16 4SB

DYNAMIC SEPARATION, WEAR AND DEFORMATION OF METAL-ON-POLYETHYLENE BEARINGS UNDER VARIATIONS IN COMPONENT POSITIONING
M. Ali, M. Al-Hajjar, LM. Jennings, J. Fisher
Institute of Medical and Biological Engineering, School of Mechanical Engineering, University of Leeds, Leeds, UK

WEAR ASSESSMENT OF METAL-ON-METAL CERVICAL TOTAL DISC REPLACEMENT UNDER STANDARD ISO TESTING PROTOCOL.
K.M.Pasko, R.M.Hall, A. Neville, J.L. Tipper
School of Mechanical Engineering/Biomedical Sciences, University of Leeds, Leeds, United Kingdom.



Tuesday 6th September

Mid - Morning Session: Debates Chamber

11:50 – 13:00 FREE PAPER SESSION: CLINICAL OSTEOARTHRITIS CONTINUED…

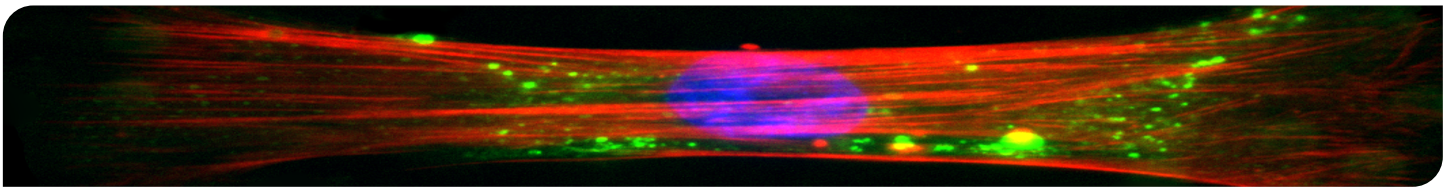
IMPACT OF OBESITY ON PATIENT-REPORTED OUTCOMES FOLLOWING TOTAL KNEE ARTHROPLASTY
DF Hamilton, K Giesinger, JM Giesinger, FL Loth, AHRW Simpson, CR Howie
Department of Orthopaedics, University of Edinburgh, 49 Little France Crescent, Edinburgh, EH16 4SB.

DAMAGE MODE ANALYSIS OF 22 AES TOTAL ANKLE REPLACEMENT EXPLANTS.
A. A. Stratton-Powell, J. L. Tipper, S. D. Williams, A. Redmond, C. L. Brockett.
Institute of Medical and Biological Engineering, University of Leeds, Leeds, UK, LS2 9JT.

SUBCHONDRAL BONE LOSSES DURING OSTEOARTHRITIS PROGRESSION
Jing Niu, Johann Henckel, Alister Hart, Chaozong Liu
Division of Surgery & Interventional Science, University College London, RNOH, Stanmore HA7 4LP, UK.

THE EFFECT OF TOURNIQUET USE ON THE DISTRIBUTION OF LOCAL ANAESTHETIC IN ADDUCTOR CANAL BLOCKS FOR TOTAL KNEE REPLACEMENT: A CADAVERIC STUDY
A. Nair, J. Dolan, K. E. Tanner, P. J. Pollock, C. Kerr, F. Barcelo Oliver, M. J. Watson, B. Jones, C. F. Kellett
Room 345 West Medical Building, University of Glasgow, Glasgow G12 8QQ.

13:00 – 14:00 LUNCH SERVED IN THE DINING ROOM



Tuesday 6th September

Afternoon Session: Debates Chamber

14:00 – 15:30 BONE MINERALISATION & OSTEOPOROSIS

CHAIRING: DR P.M. TSIMBOURI & MR D. SHIELDS

14:00 – 14:30 KEYNOTE: MANIPULATING OSSIFICATION THROUGH CHEMISTRY
PROFESSOR LIAM GROVER
Director of Research, School of Chemical Engineering, University of Birmingham.

14:30 – 15:25 FREE PAPER SESSION MINERALISATION & OSTEOPOROSIS

THE EFFECTS OF MITOCHONDRIAL DYSFUNCTION ON OSTEOBLAST FUNCTION IN THE PATHOGENESIS OF OSTEOPOROSIS.
P.F. Dobson, L.C. Greaves, D.J. Deehan, D.M. Turnbull.
Wellcome Trust Centre for Mitochondrial Research, Newcastle University, United Kingdom NE2 4HH.

OSTEOPOROSIS AND AGEING AFFECTS STEM CELL DIFFERENTIATION AND MIGRATION
A Sanghani Kerai, M J Coathup, L Osagie, S Samizadeh, G Blunn.
Institute of Orthopaedics and Musculoskeletal Science (UCL), Brockley Hill. Stanmore. HA7 4LP. London.

PTH 1-34 EFFECTS THE MIGRATION AND DIFFRENTIATION OF YOUNG AND OVARECTOMIZED BONE MARROW DERIVED RAT STEM CELLS
L. Clouard, A. Sanghani-Kerai, M. Coathup, T. Briggs, G. Blunn.
Institute of Musculoskeletal Sciences, University College London, RNOH, Stanmore HA7 4LP.

THE RELATIONSHIP BETWEEN MINERAL TO COLLAGEN RATIO, ULTRASTRUCTURE AND MECHANICAL PROPERTIES, DIFFERENCES WITHIN A SINGLE SPECIES.
P. Elston, J. Churchwell, A. Goodship, J.G. Kerns, H. Birch
Institute of Musculoskeletal Sciences, University College London, RNOH, Stanmore, HA7 4LP, UK

DEVELOPMENT OF 3D OSTEOPOROTIC MODEL FOR MICRORNA ASSESSMENT AND MANIPULATION
RK Silverwood, CC Berry, F Ahmed, RMD Meek, MJ Dalby.
Centre for Cell Engineering, University of Glasgow, Glasgow.

ARE THE CRACKS STARTING TO APPEAR IN BISPHOSPHONATE THERAPY?
SC. Ma, E.L. Goh, B. Patel, AD. Jin, O.R. Boughton, J.P. Cobb, U. N. Hansen, R.L. Abel.
Room 707, City and Guilds Building, Imperial College London, SW7 2AZ.

15:25 – 15:30 LI-COR SPONSOR PRESENTATION

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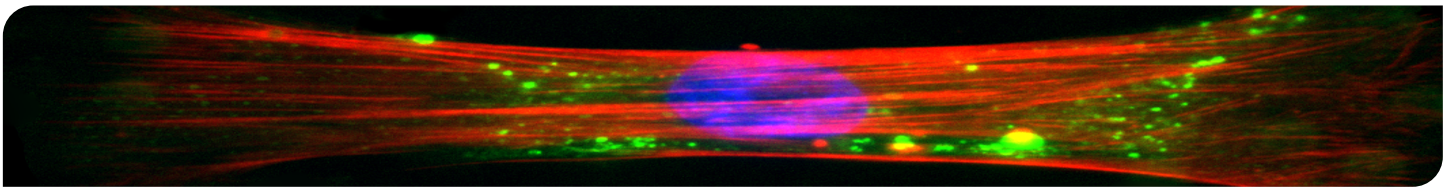
15:30 – 16:00 COFFEE AND REFRESHMENTS SERVED IN THE READING ROOM. POSTER VIEWING.



Tuesday 6th September

Late - Afternoon Session: Debates Chamber

16:00 – 16:30 INVITED PUBLIC LECTURE

CHAIRING: DR M. BIRCH & DR S COUPAUD

16:00 – 16:35 KEYNOTE: GRAVITY WAVES, STEM CELLS AND BONE REGENERATION
PROFESSOR STUART REID
Professor Experimental Physics, University of the West of Scotland.

16:35 – 16:40 PRESENTATION BORS PRIZES FOR BEST ORAL, BEST POSTER & YOUNG INVESTIGATOR

16:40 – 16:45 CLOSE OF CONFERENCE

MR RMD MEEK AND PROF MJ DALBY

PLEASE NOTE: CONFERENCE AWARDED 6 CPD POINTS FOR FULL ATTENDANCE.
ALL ORAL PRESENTATION TO BE PUBLISHED IN BJJ PROCEEDINGS IN DUE COURSE.
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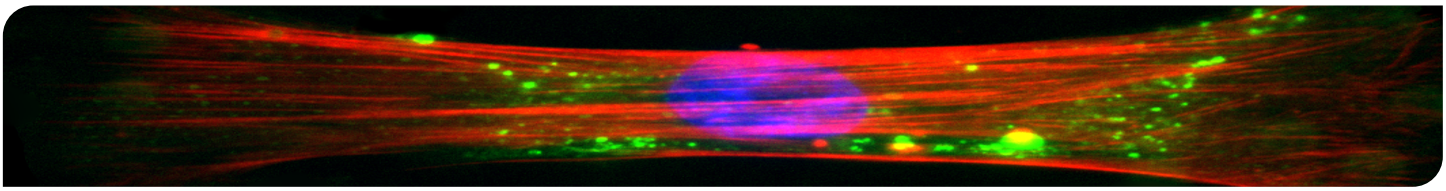

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Monday 5th September
Morning Session: Debates Chamber
STEM CELL SESSION
CHAIRING: DR R. WALLACE & MR R. SILVERWOOD

KEYNOTE: BONE REGENERATION: MAKING THE MOST OF MSC'S.

PROF P. GENEVER, DEPARTMENT OF BIOLOGY, UNIVERSITY OF YORK.
Email:

Bone marrow mesenchymal stromal cells (MSCs) are heterogeneous cell populations that are likely to contain rare stem cell subsets as well as additional, functionally-important non-stem cell fractions. We have generated several immortalised, clonal human MSC lines to study functional heterogeneity and we have identified significant variation in potency and immunoregulatory profiles. The MSC lines can be grown as 3D spheroids and genetically modified, for example using CRISPR/Cas9, to introduce insertion/deletion mutations to model and track skeletogenic programmes and disease onset/progression. This talk will cover some of our work in these areas and explain how disentangling the biological versatility of MSCs can deepen our understanding of MSC function and advance new therapeutic development particularly for bone and joint disorders.

1. **MODULATING OSTEOGENESIS IN HUMAN MSCS BY GOLD NANOPARTICLE DELIVERY OF MIR 31A ANTAGOMIRS**

MA McCully, A Vatsa, Joao Conde, Pedro Baptista, Helen Wheadon, M Dalby , C Berry
Centre for Cell Engineering, Institute of Molecular, Cell & Systems Biology, CMVLS, University of Glasgow, Glasgow.
Email: Catherine.berry@glasgow.ac.uk

MiRNAs perform gene regulation that can target approximately 60% of human protein coding genes. Along with many cellular processes, miRNAs have been implicated in stem cell differentiation. Osterix (Osx), which is inhibited by mir-31, is required by MSCs for early osteoblast differentiation resulting in bone formation further downstream. We used antagomir functionalised gold nanoparticles (AuNPs) to block mir-31, which resulted in upregulation of Osx in pre-osteoblastic MG63 cells and human mesenchymal stem cells (MSCs).

We used MG63 pre-osteoblastic cell line and human MSCs. Cytotoxicity of AuNPs was assessed by MTT, and cellular uptake of AuNPs was verified by TEM and ICP-MS. Osx RNA levels were determined by Fluidigm analysis and protein expression by In Cell Western analysis.

Antagomir-functionalised AuNPs were incubated with cells for an initial 48 hours. (1) No cytotoxic effects were noted in either cell type. (2) Fluidigm analysis identified a varied gene response to antagomir delivery in both cell types, with MSCs recording a reduction of stem cell marker genes nestin, alcam, CD63, and CD44 at day 5 (indicating differentiation). (3) Osx protein levels were increased in both cell types after 48 hour incubation. (4) Downstream MSC analysis demonstrated accelerated osteogenesis at week 3 and 5 (verified by osteocalcin nodule formation) following 48 hour AuNP incubation.

RNA analysis in both cell types suggested a shift away from proliferation towards osteoblastic differentiation. This was supported by Osx protein expression, which was increased in both MG63 cells and MSCs. Finally, an increase in the late osteogenic marker (osteocalcin) was verified at weeks 3 and 5 in MSCs after AuNP incubation for 48 hours. These results collectively infer successful delivery of mir-31 antagomirs, which are blocking mir-31-mediated suppression of Osx, resulting in an early increase in Osx, which accelerates MSC osteogenesis downstrea

2. PTH 1-34 EFFECTS THE DIFFRENTIATION OF BONE MARROW STEM CELL DERIVED ADIPOCYTES

L. Clouard, A. Sanghani-Kerai, M. Coathup, T. Briggs, G. Blunn
Institute of Musculoskeletal Sciences, University College London, Royal National Orthopaedic Hospital, Stanmore HA7 4LP
Email:

Osteoporosis is characterised by an uncoupling of bone formation and resorption resulting in net resorption. Stem cells derived from bone marrow in osteoporotic patients typically contain more adipocytes,. Intermittent Parathyroid hormone (iPTH), has been shown to cause the preferential differentiation of mesenchymal stem cells (MSCs) to osteoblasts. We isolated rat bone marrow derived MSCs, investigating the effect of iPTH on adipocyte differentiation.

MSCs were harvested from the femora of 6-10week oldWT rats and cultured to induce adipogenesis for 21 days. Subsequently, cells were continually cultured in adipogenic media, osteogenic media or in osteogenic media supplemented with PTH 1-34 either continuously or intermittently for 6hours in every 72hour cycle. ALP and Alizarin Red assessed osteogenic differentiation, and Oil Red O used to assess intracellular microdroplet formation.A student t-test was used to analyse results, and a p value<0.05 considered significant.

Quantitatively measurements of Alizarin Red staining significantly increased in all adipocytes grown in osteogenic media compared to the cells continually cultured in adipogenic media.Calcium phosphate deposition continued to increase significantly in these groups up to day 14. At day 14, Alizarin Red staining from cells cultured in iPTH were significantly higher than osteogenic media alone.

ALP expression was significantly higher for cells cultured in osteogenic media and iPTH compared to adipogenic media at days 3-14. Expression peaked at day 7, at this timepoint cells cultured in iPTH expressed significantly more ALP than other groups (Figure 2). Oil Red O measurements were significantly reduced from days 7-14 for all osteogenic groups, this significance was greatest for the iPTH group at day 7.

iPTH increased the transdifferentiation of adipocytes derived from MSCs into osteoblasts, this effect was most significant after 7 days. Ultimately, the role of iPTH on adipocytes may lead to improved bone formation with many orthopaedic applications.

3. **THE MAGNETIC LABELLING OF STEM CELLS TO ENGINEER THE BONE MARROW NICHE *IN VITRO***

N. Lewis, E. Lewis, M. Dalby & C. C. Berry
Centre for Cell Engineering, Joseph Black Building, University Avenue, University of Glasgow, Glasgow
Email: n.lewis.1@research.gla.ac.uk

Hematopoietic stem cells (HSCs) reside within a specialised niche area in the bone marrow (BM). They have tremendous clinical relevance, although HSC expansion and culture *ex vivo* is not currently possible, reducing BM transplant success. This project expands a novel 3D MSC niche model developed in our lab to include HSCs.

MSCs were loaded with green fluorescent magnetic iron oxide (FeO_3) nanoparticles (200 nm diameter) at a concentration of 0.1 mg ml^{-1}, and incubated for 30 min over a magnet to enhance cellular uptake. The cells were washed, detached and resuspended, then transferred to a plate with magnets above. Spheroids formed within hours and were implanted into 2 mg ml^{-1} collagen gel. HSCs were loaded with nanoparticles via incubation with suspension, and then introduced to the gel containing the spheroid. Immunostaining, BrdU and Calcein/ ethidium homodimer viability assays were performed to characterise the cells.

Cells in both monolayers and spheroids remain viable up to 7 days in culture. MSCs in monolayers and spheroids were stained with antibodies for: STRO-1, an MSC marker; SDF-1 (CXCL-12), a secreted HSC homing factor; and nestin, a marker for HSC-supportive MSCs *in vitro.* MSCs in spheroids retain a higher level of expression of all three for 7 days compared to MSCs in monolayers. BrdU assay results show that the MSCs are more quiescent in spheroids compared to monolayers.
Proof of principle studies are promising for the success of the proposed niche model. MSCs express a higher level of MSC markers and retain quiescence when they are in spheroids as compared to monolayers. They also express a higher level of HSC niche factor SDF-1α, which facilitates HSC migration and retention.

4. DIFFERENCES IN MORPHOLOGY, PROLIFERATION AND IMMUNEPROFILE AMONG SINGLE-CELL CLONED STEM CELLS FROM THE SAME MESENCHYMAL STEM CELL ORIGIN

Y. Cheng, S. Sorousheh, M. Coathup, G. Blunn
John Scales Centre for Biomedical Engineering, Institute of Orthopaedics and Musculoskeletal Science, Division of Surgery and Interventional Science, Royal National Orthopaedic Hospital University College London, Brockley Hill, Stanmore, Middlesex HA7 4LP
Email:

Mesenchymal stem cells (MSCs) are usually believed to be immune-privileged. However, immunogenic MSCs were also reported. We hypothesize that there are differences between MSC clones from the same individual in terms of their morphology, proliferation, differentiation and immunogenicity. Our goal is to discover immune-privileged stem cells for universal allogenic MSCs transplantation.
Serial dilutions of bone-marrow derived (BMMSCs) and adipose derived mesenchymal stem cells (ADMSCs) from same animal were carried out to isolate single-cell clones. From a single animal we obtained 3 clones from BMMSCs and 3 from ADMSCs. The proliferation rate of each clonal culture and mixed clonal culture were measured. The tri-differentiation potential of the clonal cultures was compared, as well as with the original isolates from bone marrow and fat. The immune-privileged properties were measured by flow cytometry and immuno-staining for the major histocompatibility complex (MHC) antigens. Mixed leucocyte reaction (MLR) were also performed to investigate immunogenicity.
Tri-differentiation was confirmed in all isolates. All clonal cultures revealed significant different morphology and proliferation rates, compared with each other and mixed cultures. All clonal cultures showed different surface markers, inclusive of MHC antigens. One clone from ADMSCs showed lack of MHC antigens. Our MLR and MHC staining disclosed variety of immune properties.
All clones tri-differentiated which indicated a degree of 'stemness'. MSCs are generally believed not to express MHC II, resulting in immune-privileged. Our results confirmed our hypothesis because clonal cultures isolated from different origins of same animal show differences in morphology, proliferation rate, and surface marker presentation. Individual immune differences highlighted through single-cell clonal cultures may be crucial to find universal immune-privileged MSCs as universal allogeneic donor.

5. MATERIAL-DRIVEN FIBRONECTIN NANONETWORKS AS EFFICIENT BMP2 MICROENVIRONMENTS FOR BONE REPAIR

C. Gonzalez-Garcia[1], V. Llopis-Hernandez[1], D. Shields, M. Cantini[1], A. Alba[1], A.J. Garcia[2], M. Dalby[3], M. Salmeron-Sanchez[1]
[1] Biomedical Engineering Research Division, School of Engineering, University of Glasgow. UK. [2] Woodruff School of Mechanical Engineering and Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, US. [3] Center for Cell Engineering, Institute of Biomedical and Life Sciences, University of Glasgow, UK
Email: Manuel.Salmeron-Sanchez@glasgow.ac.uk

Material-based strategies seek to engineer synthetic microenvironments that mimic the characteristics of physiological extracellular matrices for applications in regenerative therapies, including bone repair and regeneration. In our group, we identified a specific chemistry, poly(ethyl acrylate) (PEA), able to induce the organization of fibronectin (FN), upon adsorption of the protein, into fibrillar networks similar to the physiological ones, leading to enhanced cellular response, in terms of cell adhesion and differentiation. In this work, we exploit these FN networks to capture and present growth factors (GF) in combination with the integrin binding domain of FN during bone tissue healing.

Fibrillar conformation of FN adsorbed on PEA favors the simultaneous availability of the GF binding domain (FNIII12-14) next to the integrin binding region (FNIII9-10), compared to poly(methyl acrylate) (PMA), a material with similar chemistry, where FN adopts a globular conformation. The combined exposure of specific adhesive sequences recognized by integrins and GF binding domains was found to improve the osteogenic differentiation of mesenchymal stem cells. A higher expression of bone proteins was found when BMP2 is bound or sequestered on the material surface versus its administration in the culture media in vitro. The potential of this system as recruiter of GFs was also investigated in a critical-size bone segmental defect in mouse. The synergistic integrin-GF signalling, induced by fibrillar FN, promoted bone formation in vivo with lower BMP2 doses than current technologies. Furthermore, we optimized the system for its potential use in translational research, seeking to address the clinical need of using biocompatible and biodegradable material implants. Polycaprolactone scaffolds were synthesized and coated with a thin layer of plasma- polymerized PEA that recruits and efficiently presents GF during healing of critical size defects.

The material-driven FN fibrillogenesis provides a new strategy to efficiently reduce the GF doses administrated in bone regenerative therapies.

6. USING SURFACE CHEMISTRY TO REGULATE BEHAVIOUR OF MESENCHYMAL STEM CELLS

P Sweeten[1,2], R Gurden[1,2], L-A Turner[1], E Ross[3], M. Salmeron-Sanchez[2], J Mountford[3], M.J. Dalby[1]
[1] Centre for Cell Engineering, Institute of Molecular, Cell, and Systems Biology, University of Glasgow, UK [2] Microenvironments for Medicine, Division of Biomedical Engineering, University of Glasgow, UK [3] Institute of Cardiovascular and Medical Sciences, , University of Glasgow, UK
Email: matthew.dalby@glasgow.ac.uk

Control of stem cell fate and function is critical for clinical and academic work. By combining surface chemistry-driven extracellular matrix (ECM) assembly with mesenchymal stem cells (MSCs) we are developing a system which can be used to regulate the behaviour of MSCs. The conformation of the ECM glycoprotein fibronectin (Fn) is different when adsorbed onto poly methylacrylate (PMA) where it is globular, and on poly ethylacrylate (PEA) where it forms a physiologically-similar network[1] (Fig. 1). Using these polymers to govern Fn conformation, we are developing a 3D system incorporating MSC-responsive growth factors (GFs) and bone marrow MSCs capable of regulating MSC behaviour.

Toluene-dissolved PMA and PEA were spin coated onto glass coverslips before solvent extraction *in vacuo* and UV sterilisation. 20 mg ml^{-1} human plasma FN was adsorbed onto the surfaces followed by 25 ng ml^{-1} recombinant human BMP2/VEGF. FN conformations were characterised by atomic force microscopy (AFM). A collagen hydrogel was placed above the substrate. Adult human bone marrow STRO-1+ were cultured on the substrates for 3 weeks in supplemented DMEM. Expression of MSC stemness and HSC maintenance factors were analysed by In-Cell Western assay.

To establish the best combination of polymer/FN/GF, MSC stemness markers (ALCAM, NESTIN and STRO1), osteogenic differentiation markers (OCN and OPN) and bone marrow markers (SCF and VCAM1) were measured in MSCs cultured for 3-weeks on substrates. OCN, SCF, and VCAM1 expression was enhanced across all combinations compared to glass control, while for ALCAM/STRO1/NESTIN and OPN, PEA combinations enhanced their expression.

PEA + FN + VEGF appeared to be system best suited to maintaining MSC stemness and supporting expression of osteogenesis markers and bone marrow markers.

We have shown that MSCs maintain their stem cells state and express high levels of SCF and VCAM-1 when cultured on PEA with adsorbed Fn and VEGF or BMP2. Next stages of this work will use PCR to verify results and analyse expression of other MSC markers to develop a role for these synthetic polymers as biomaterials.

[1]Gonzalez-Garcia C, Moratal D, Oreffo RO, Dalby MJ, Salmeron-Sanchez M. Integrative biology : quantitative biosciences from nano to macro.

Mid - Morning Session: Debates Chamber

BIOMATERIALS: BONE REMODELLING

CHAIRING: PROF M. WILKINSON & DR M. CANTINI

KEYNOTE: ENGINEERED HYDROGELS FOR CELL DELIVERY AND BONE REPAIR

PROF A. GARCÍA
Rae S. and Frank H. Neely Chair and Regents' Professor Woodruff School of Mechanical Engineering. Georgia Institute of Technology, USA.
Email:

Hydrogels, highly hydrated cross-linked polymer networks, have emerged as powerful synthetic analogs of extracellular matrices for basic cell studies as well as promising biomaterials for regenerative medicine applications. A critical advantage of these artificial matrices over natural networks is that bioactive functionalities, such as cell adhesive sequences and growth factors, can be incorporated in precise densities while the substrate mechanical properties are independently controlled. We have engineered poly(ethylene glycol) [PEG]-maleimide hydrogels for the controlled delivery of therapeutic proteins and hum an mesenchymal stem cells to heal critical-sized segmental bone defects in mice. Materials engineering to present integrin-specific, cell-adhesive peptides enhanced mesenchymal stem cell survival and engraftment and resulted in improved bone repair. Additionally, these materials can be engineered to reduce bacterial infections in the context of bone repair. These studies establish these engineered PEG hydrogels as promising biomaterial carriers for cell delivery, engraftment and enhanced tissue repair.

7. PHOSPHONATE-TETHERED LYSOPHOSPHATIDIC ACID-FUNCTIONALISED TITANIUM: A NOVEL SURFACE FINISH FOR CEMENTLESS ORTHOPAEDIC IMPLANTS.

W Nishio Ayre[1], T Scott[2], K Hallam[2], AW Blom[3], SP Denyer[4], H Bone[5], JP Mansell[6]
[1]School of Dentistry, Cardiff University, Cardiff CF14 4XY, UK; [2]Interface Analysis Centre, School of Physics, University of Bristol, Bristol BS8 1TL, UK; [3]Musculoskeletal Research Unit, University of Bristol, Southmead Hospital, BS10 5NB, UK; [4]School of Pharmacy and Biomolecular Sciences, University of Brighton, BN2 4AT; [5]CATIM, University of the West of England, BS16 1QY ; [6]Department of Biological, Biomedical & Analytical Sciences, University of the West of England, BS16 1QY
Email:

In England and Wales in 2012 over 160,000 primary total hip and knee replacements were performed with 57% of hip replacements utilising uncemented prostheses. The main cause of failure, affecting approximately 10% of patients, is aseptic loosening. Previous research has found that functionalising titanium with lysophosphatidic acid (LPA) induces an increase in human osteoblast maturation on the implant surface through co-operation with active metabolites of vitamin D3. This feature, the small size of the LPS molecule and its affinity to readily bind to titanium and hydroxylapatite makes it an especially desirable molecule for bone biomaterials. Nevertheless biomaterials that also demonstrate anti-microbial properties are highly desirable.

To test the antimicrobial efficacy of the LPA-functionalised titanium, a clinical isolate of *Staphylococcus aureus,* obtained from an infected revision surgery, was cultured on the surface of titanium discs functionalised with 0, 0.1. 0.5, 1, 2 and 5µM LPA. Bacterial adhesion was quantified at 1, 2, 6, 12 and 24 hours by live/dead counts and biofilm mass quantified by crystal violet staining after 24, 48, 72 and 96 hours culture. To elucidate the mechanisms of action of LPA, proteomic analysis of adhered bacteria was performed using SDS-PAGE and Western blots.

500nM to 1µM LPA were the optimum concentrations to significantly inhibit bacterial adhesion (ANOVA, $p<0.001$). These concentrations also reduced biofilm mass on the surface of the titanium. Proteomic analysis highlighted an increase in low molecular weight proteins as a result of optimal LPA surface concentrations. Fatty acid chains as found in LPA have previously been associated with causing leakage of low molecular weight proteins through increased cell membrane permeability.

LPA coatings have the potential to enhance implant osseointegration whilst simultaneously reducing bacterial attachment. This technology may reduce both septic and aseptic failure of cementless joint prostheses, ultimately prolonging implant longevity and patient quality of life.

8. PRECISION ENGINEERED NANOTOPOGRAPHICAL SURFACES TO CONTROL OSTEOCLAST DIFFERENTIATION

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Recent studies have shown that random disorder nanotopographical surfaces increase osteoblast differentiation and bone formation. This has great potential merit in spinal fusion surgery and arthroplasty, however, osteoclast mediated osteolysis is responsible for the aseptic failure of implanted biomaterials, and there is a paucity of literature regarding osteoclast response to nanoscale surfaces. Furthermore, imbalance in osteoclast/osteoblast resorption is responsible for osteoporosis, a major healthcare burden. We aimed to assess the affect of nanopatterned surfaces on osteoclastogenesis.

We cultured CD14/16+ monocytes (osteoclast precursors) on random disordered nanopatterned (NSQ50), ordered nanopatterned (SQ) and unpatterned polycarbonate substrates. We also developed a novel human osteoblast/osteoclast co-culture system without extraneous supplementation to closely represent the *in vivo* environment. We assessed the cultures using electron microscopy (SEM), protein expression using immunofluorescence and histochemical staining and gene expression using polymerase chain reaction (PCR).

In complex co-culture significantly increased osteoblast differentiation and bone formation was noted on disordered nanotopography, suggesting improved osteointegration. In isolated culture mature osteoclast like cells were seen on plain and SQ but not on NSQ50 surfaces, suggesting specific anti-osteoclastogenic properties of this surface.

Our results show that osteoclast differentiation can be controlled through use of random disorder nanopatterned surface features. We also confirmed successful co-culture of osteoblasts and osteoclasts using a unique method closely resembling the *in vivo* environment encountered by orthopaedic implants, with increased bone formation. This offers great potential in biomaterial design and may offer potential targets for osteoporosis therapy.

9. DEVELOPMENT OF AN ADAPTIVE BONE REMODELING MODEL DRIVEN BY MECHANICAL AND BIOLOGICAL STIMULI FOR IMPLANT ANALYSIS

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Long-term survival of massive prostheses used to treat bone cancers is associated with extra-cortical bone growth and osteointegration into a grooved hydroxyapatite coated collar positioned adjacent to the transection site on the implant shaft [1]. The survivorship at 10 years reduces from 98% to 75% where osteointegration of the shaft does not occur. Although current finite element (FE) methods successfully model bone adaption, optimisation of adventitious new bone growth and osteointegration is difficult to predict. There is thus a need to improve existing FE models by including biological processes of osteoconduction and osteoinduction.

The principal bone adaptation criteria is based on the standard strain-energy remodeling algorithm, where the rate of remodeling is controlled by the difference in the stimulus against the reference value [3]. The additional concept of bone connectivity was introduced, to limit bone growth to neighbouring elements (cells) adjoining existing bone elements. The algorithm was developed on a cylindrical model before it was used on an ovine model.

The geometry and material properties from two ovine tibiae were obtained from computed tomography (CT) scans and used to develop FE models of the tibiae implanted with a grooved collar. The bones were assigned inhomogeneous material properties based on the CT grey values and typical ovine walking load conditions were applied. The FE results show a region of bone tissue growth below the implanted collar and a small amount of osteointegration with the implant, which is in good agreement to clinical results. Some histological results suggest that further bone growth is possible and potential improvements to the model will be discussed. In summary, by including an algorithm that describes osteoconduction, adventitious bone growth can be predicted.

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[2] Huiskes, R., *et. al.* J Biomech. 1987;20(11-12):1135-50.
[3] McDonald, D. J Bone Joint Surg Am. 2013 Sep 4;95(17):e1281-2.

10. 3D PRINTED PLA SCAFFOLDS FOR BONE TISSUE REGENERATION: EFFECT OF SCAFFOLD STRUCTURE ON ATTACHMENT AND GROWTH OF HUMAN DENTAL PULP STROMAL CELLS (HDPSCS)

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Poly-lactic acid (PLA) scaffolds are widely used in bone tissue engineering. The introduction of 3D printing has greatly increased the ability for tailoring different geometrical designs of these scaffolds for improved cellular attachment, growth and differentiation. This study aimed to investigate the effect of PLA fibre angle in 3D printed PLA scaffolds on hDPSC attachment and growth *in vitro*.

Two types of PLA scaffolds were prepared via 3D printing containing fibres angled at either 45^{o} or 90^{o}. hDPSCs (P4, $2*10^{5}$ cells per scaffold) were statically seeded for 4 hours on to the scaffolds (7x3.5x3 mm^{3}, n=3). Cellular attachment was checked using fluorescence microscopy and the number of unattached cells was counted using a haemocytometer (HCM). The cell-scaffold constructs were then cultured in osteogenic medium for up to 5 weeks. ALP staining and SEM were performed for one construct from each group at week 3. Cellular viability was determined using CMFDA/EHD1 live/dead labelling at week 4. After 5 weeks, constructs were processed for histology.

Fluorescence micrographs showed high numbers of hDPSCs attached to scaffold surfaces in both groups after seeding irrespective of fibre angle. However, HCM cell count revealed that the 45° angled PLA scaffolds had significantly greater cell attachment compared to the 90° angled PLA group ($p<0.0001$). After 3 weeks in osteogenic culture, both types of construct showed strong ALP staining. SEM showed that in the 45° angled PLA group, almost all macro-pores were fully closed with newly formed cell sheets. In comparison, in the 90° angled group, most of the macro-pores remained open although a limited amount of cellular bridging was present. SEM also detected crystal deposits in different areas within the cell sheets for both construct groups. Most hDPSCs were alive in both groups at week 4 of culture with few dead cells present. After 5 weeks, histology showed marked cellular growth and new matrix formation, with detectable Van Kossa +ve crystal deposits in different areas within all constructs irrespective of PLA fibre angle.

This study showed that 45° angled PLA 3D printed scaffolds enhanced hDPSC attachment and cellular bridging, which may help to rapidly close the macro-pores within the scaffold compared to the 90° angled group. This illustrates the potential of 45^{o} angled 3D printed PLA scaffolds as good candidates for bone tissue engineering.

11. ENGINEERING OSTEOGENIC COATINGS ON PEEK

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Polyether ether ketone (PEEK) has been increasingly employed as biomaterials for trauma, orthopeadic, and spinal implants. However, concern has been raised about the inertness of PEEK which limits bone integration. In this study, we have coated PEEK with a functional material seeking to promote osteogenic differentiation of human mesenchymal stem cells (hMSC).

We have used spray drying to coat poly(ethyl acrylate) (PEA) as a coating on PEEK. This technique is simple, allows a range of controlled coating thicknesses (from hundred nm to a few um), cost effective and easily translatable to scaffolds or implant surfaces for existing or new orthopaedic applications. PEA induces the organisation of fibronectin (FN) into nanonetworks upon simple adsorption from protein solutions. These FN nanonetworks on PEA represent a microenvironment for efficient growth factor binding and presentation in very low but effective doses. In this study we show cell adhesion and stem cell differentiation towards an osteogenic lineages when bone morphogenetic protein 2 (BMP2) was adsorbed on these engineered PEEK/PEA/FN microenvironments in very low doses.

Overall, the developed functional coatings on PEEK has the potential to allow the translation of this material into orthopaedic applications.

12. GROWTH BY STRETCH: AN INTERDISCIPLINARY APPROACH TO IMPROVE CURRENT PRACTICE

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Tissue expansion is a technique used by plastic and restorative surgeons to cause the body to grow additional skin, bone or other tissues. For example, distraction osteogenesis has been widely applied in lower limb surgery (trauma / congenital), and congenital upper limb reconstruction (e.g. radial dysplasia). This complex and tightly regulated expansion process can thus far only be optimised by long-term animal or human experimentation.

Here the intent is to develop an in vitro model of tissue expansion that will allow to both optimise the extension regime (µm/h, continuous/ intermittent) and investigate using proteomic techniques which molecular pathways are involved in its regulation. Cells cultured onto sheets of polymer (PCL) can be stretched at very low, adjustable speeds, using a stepper motor and various 3D printed and laser cut designs. The system utilises plastic flow of the polymer, enabling the material to stay extended upon strain being released.
Tensile tests have displayed the plastic behaviour of the polymer sheet when stretched, and digital image correlation (DIC) has been used to analyse homogeneity of the strain field. Further analysis involving nuclear localisation of yes-associated protein (YAP) aims to link cell response to this strain field.
Nuclear orientation analysis has demonstrated a morphological response to strain (1 mm/day) in comparison to not being stretched, and this is in the process of being linked to nanoscale changes of the substrate (using atomic force microscopy) during the stretching regime. Future work will identify how strain is affecting the cell cycle, before a mass tagging approach is used to identify protein changes induced by strain.

Mid - Morning Breakout Session: Bridie Library
MOLECULAR TENDONOPATHY: TOWARD TRANSLATION

CHAIRING: MR N.L. MILLAR
INVITED LECTURE: INFLAMMATION, ACTIVATION AND RESOLUTION IN TENDONOPATHY
DR STEPHANIE G DAKIN,
UNIVERSITY OF OXFORD

13. PROTEINASE-ACTIVATED RECEPTOR 2: POTENTIAL ROLE IN OSTEOARTHRITIC ENTHESEAL PATHOLOGY

Ortiz, L. Dunning, C. Huesa, W.R. Ferrell, I.B. McInnes, J.C. Lockhart, N.L. Millar, C.S. Goodyear, A. Crilly
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Osteoarthritis (OA) is no longer considered a cartilage-centric disease with remodelling of other joint tissues now recognized. While understudied, entheseal pathology is considered a secondary OA feature. A pivotal role for proteinase-activated receptor 2 (PAR2) in OA has been demonstrated previously in cartilage and subchondral bone at early time points, however the entheseal role of PAR2 has not been reported.

OA was induced by destabilization of the medial meniscus (DMM) in wild type (WT) and PAR2 deficient (KO) animals. At 4 weeks and one year post surgery, knee joints were harvested for histological analysis. Medial collateral ligament (MCL) width was measured by 2D planimetry analysis. Immunohistochemistry was used to characterize the MCL and anterior cruciate ligament (ACL). Data were expressed as mean±SEM (n=4-6/group) and analysed using Student's t-test, with $p<0.05$ as the criterion of significance.

MCL width increased between 4 weeks and 1 year in WT DMM (0.24 ±0.07 vs 0.40 ±0.008mm respectively, $p<0.001$). Interestingly, a significant reduction in MCL was observed in KO compared with WT at 1 year (0.23 ±0.005 vs 0.40 ±0.008mm respectively, $p <0.001$) post-DMM. Further characterization of DMM WT MCL and ACL at 4 weeks showed the presence of $F4/80^+$ cells in addition to IL-33 and histamine. At one year post-surgery, a cellular infiltrate was observed in MCL DMM WT but absent in KO mice. Histological evaluation revealed an absence of $F4/80^+$ cells but the presence of a $PAR2^+$ population, subsequently identified as hypertrophic-like chondrocytes (RUNX2) and chondrocytes-like cells (SOX9).

Deletion of PAR2 affords long-term protection against ligament remodelling and demonstrates a critical role for this receptor in both OA joint pathology and ligament injuries. While PAR2 appears to be a credible therapeutic target in OA entheseal pathology, further understanding of the molecular mechanism regulated by this receptor will be required.

14. DEVELOPING A NOVEL 3-DIMENSIONAL (3D) CO-CULTURE SYSTEM TO GENERATE A TENDON-BONE TISSUE INTERFACE: EVALUATION OF HYDROGELS

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Entheses are the anchorage sites of tendons to bones in the musculoskeletal system. They have a unique microanatomy that allow smooth transfer of mechanical load through tendon to bone. However, entheses are prone to injury due to their small surface area[1,2]. The overall success rate of the current gold standard treatment (directly attaching the tendon to bone) is small[3,4]. Consequently, the aim of this study was to evaluate different hydrogels and their suitability for developing an *in-vitro* co-culture system to manufacture 3D tissue interfaces.

To create a 3D *in-vitro* tissue interface, half-well plugs were created by pouring silicone in wells of a 24-well plate. When set, it was cut into halves to be used as half-well plugs, blocking one side of a culture well. A tendon-cell-encapsulated hydrogel was poured into the exposed half and, when set, the plug was removed and a bone-cell-encapsulated gel was added. Cells were fluorescently labelled to enable identification of cell types under fluorescent microscopy (Tendon – green, bone – red). The suitability of different hydrogels to form an *in vitro* tissue interface was evaluated: fibrin, agarose and gellan.

This study demonstrates that 3D co-cultures can be manufactured *in-vitro*. The novel system enabled the culture of two cell types (bone/tendon) in direct contact, creating an *in-vitro* interface. In addition, this study shows that fibrin gel supports cell morphology, while both cell types failed to show normal morphology in agarose and gellan. Further studies evaluating cell viability in these hydrogels are currently underway.

1. Paxton, J. Z. et al. *Orthop. Muscular Syst.* **S1,** (2012).
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3. Beitzel, K. *et al. Arthrosc. - J. Arthrosc. Relat. Surg.* **29,** (2013).
4. Lafosse, L. Surgical technique. *J. bone Jt. Surg. Am. Vol.* **90,** (2008).

INVITED LECTURE: PROTEOMIC AND TRANSCRIPTOMIC SIGNATURES IN TENDON DISEASE
DR MANDY PEFFERS, UNIVERSITY OF LIVERPOOL

15. THE EFFECTS OF TERMINAL STERILISATION USING VARYING IRRADIATION DOSAGES ON THE BIOMECHANICAL PROPERTIES OF THE ACELLUAR PORCINE FLEXOR TENDON

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Acellular porcine super flexor tendon (pSFT) offers a promising solution to replacement of damaged anterior cruciate ligament [1]. It is desirable to package and terminally sterilise the acellular grafts to eliminate any possible harmful pathogens. However, irradiation techniques can damage the collagen ultra-structure and consequently reduce the mechanical properties [2]. The aims of this study were to investigate the effects of irradiation sterilisation of varying dosages on the biomechanical properties of the acellular pSFT.

Tendons were decellularised using a previously established protocol [1] and subjected to irradiation sterilisation using either 30 kGy gamma, 55 kGy gamma, 34 kGy E-beam, 15 kGy gamma, 15 kGy E-beam and (15+15) kGy E-beam (fractionated dose). Specimens then underwent stress relaxation and strength testing at 0 and 12 months post sterilisation to determine whether any effect on these properties was progressive. For stress relaxation testing, specimens were analysed using a Maxwell-Wiechert model. For strength testing, the ultimate tensile strength, Young's modulus and failure strain were assessed.

Significant differences were found which demonstrated that all irradiation treatments had an effect on the time-independent and time-dependent viscoelastic properties of irradiated tendons compared to per-acetic acid only treated controls. Interestingly, no significant differences were found between the irradiated groups. Similar trends were found for the strength testing properties. No significant differences were found between groups at 0 and 12 months.

Tendons retained sufficient biomechanical properties following sterilisation, however it was notable that there were no significant differences between the irradiated groups, as it was believed higher dosages would lead to a greater reduction in the mechanical properties. The changes observed were not altered further after 12 months storage, indicating the acellular pSFT graft has a stable shelf-life.

References:

1. Herbert et al. (2015). J Biomechanics. 48 p22-29.
2. Yanke et al. (2013). Am J Sports Med. 42(4), p835-840.

16. S100 PROTEINS: DANGER SIGNALS IN TENDINOPATHY

Lindsay A N Crowe[1], Moeed Akbar[1], Susan M Kitson[1], James H Reilly[1], Shauna C Kerr[1], George A C Murrell[2], Iain B McInnes[1], Derek S Gilchrist[1], Neal L Millar[1];
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Alarmins- also referred to as damage associated molecular patterns (DAMPS)- are endogenous molecules mobilized in response to tissue damage known to activate the innate immune system and regulate tissue repair and remodelling. The molecular mechanisms that regulate inflammatory and remodelling pathways in tendinopathy are largely unknown therefore identifying early immune effectors is essential to understanding the pathology. S100A8 and S100A9 are low molecular weight calcium binding proteins primarily released by activated phagocytes in an inflammatory setting and also secreted as a heterodimeric complex that exhibits cytokine like functions. Based on our previous investigations we sought evidence of S100A8/A9 expression in human tendinopathy and thereafter, to explore mechanisms whereby S100 proteins may regulate inflammatory mediators and matrix regulation in human tenocytes.

Torn supraspinatus tendon (established pathology) and matched intact subscapularis tendon (representing 'early pathology') biopsies were collected from patients undergoing arthroscopic shoulder surgery. Control samples of subscapularis tendon were collected from patients undergoing arthroscopic stabilisation surgery. S100A8/A9 expression was analysed at transcript and protein level using quantitative RT-PCR and immunohistochemistry, respectively. Primary human tenocytes were cultured from hamstring tendon tissue obtained during hamstring tendon ACL reconstruction. The *in vitro* effect of recombinant human S100 A8/A9 on primary human tenocytes was measured using quantitative RT-PCR and ELISA.

Immunohistochemistry of tendinopathic tissues demonstrated the presence of S100 A8/A9 in diseased tissues compared to control tissue. In addition, early pathological diseased tissue indicated greater S100A9 expression compared with established diseased pathology. These findings were reflected by data obtained at transcript level from diseased tissues. Recombinant human S100A8, A9 and A8/A9 complex led to significant increase in expression of inflammatory mediators, including IL-6 *in vitro*. Further analysis via quantitative RT-PCR demonstrated recombinant S100A8, A9 and A8/A9 complex treatment on tenocytes, *in vitro*, had no direct effect on the expression of genes involved in matrix remodelling.

The presence of S100A8 and S100A9 in early tendinopathic lesions suggests expression is upregulated in response to cellular damage. S100A8 and S100A9 are endogenous ligands of Toll-like receptors (TLRs) and receptor for advanced glycation end products (RAGE). These receptors have known regulatory effects on immune mediated cytokine production. We propose S100A8 and S100A9 as active alarmins in the early stages of tendinopathy and thus targeting of its downstream signalling may offer novel therapeutic approaches in the management of human tendon disorders.

1. Millar, N. L. *et al.* MicroRNA29a regulates IL-33-mediated tissue remodelling in tendon disease. *Nat Commun* **6**, 6774 (2015).

INVITED LECTURE: NEURAL PATHWAYS IN TENDONOPATHY
BJF DEAN, UNIVERSITY OF OXFORD

Afternoon Session: Debates Chamber
PRECISION ORTHOPAEDICS AND ADVANCED MANUFACTURING

CHAIRING: MR M. BLYTH & DR I. SMITH
KEYNOTE: ROBOTS IN KNEE OA: A COMPARTMENTALISED SOLUTION?

MR MARK BLYTH
Orthopaedic Consultant, Glasgow Royal Infirmary, Glasgow, UK.

17. CREATING THE NEXT GENERATION OF ORTHOPAEDIC FIXATION SCREWS – A NOVEL USE OF 3-D PRINTING

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Currently available fracture fixation devices that were originally developed for healthy bone are often not effective for patients with osteoporosis. Resulting outcomes are unsatisfactory, with longer recovery times, often requiring re-surgery for failed cases. One major issue is the design of bone screws, which can loosen or

pull-out from osteoporotic bone. Design improvements are possible, but the development of new screws is a lengthy and expensive process due to the manufacture of the complex geometry involved. The aim of this research was to validate our currently available 3D printing technology in the design, manufacture and testing of screws.

Three standard wood screw designs were reverse-engineered using computational modelling and then fabricated in polymeric resin using 3D rapid prototyping on a Stereolithography (SLA) machine. The original metal screws and the 3D screws (n=5 of each) were then inserted into a synthetic bone block (Sawbones, PCF5) representing the mechanical properties of severely osteoporotic cancellous bone. Pull-out tests were conducted in accordance with ASTM 543-13.

The three metal screws exhibited pull-out strengths of 125, 74 and 118 N respectively. The 3D printed screws by comparison showed pull-out strengths approximately 15-20 % lower than their metal counterparts. However, when the results were normalised to the material tested, showing the relative changes to the first design, the pattern of results in the metal and 3D printed groups were almost identical (within 3 % of each other), showing excellent correlation.

This study is the first to show that 3D Rapid Prototyping can be used in the pre-clinical testing of orthopaedic screws. The methodology provides a cheaper, faster development process for screws, allowing huge scope for development and improvement. Future work will include expanding the study to include more screw configurations as well as testing in higher density foams to compare performance in healthier bone.

18. **DEVELOPMENT OF AN AUGMENTED REALITY-GUIDED COMPUTER ASSISTED ORTHOPAEDIC SURGERY SYSTEM**

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A number of advantages of unicondylar arthroplasty (UKA) over total knee arthroplasty in patients presenting osteoarthritis in only a single compartment have been identified in the literature. However, accurate implant positioning and alignment targets, which have been shown to significantly affect outcomes, are routinely missed by conventional techniques. Computer Assisted Orthopaedic Surgery (CAOS) has demonstrated its ability to improve implant accuracy, reducing outliers. Despite this, existing commercial systems have seen extremely limited adoption. Survey indicates the bulk, cost, and complexity of existing systems as inhibitive characteristics. We present a concept system based upon small scale head mounted tracking and augmented reality guidance intended to mitigate these factors.
A visible-spectrum stereoscopic system, able to track multiple fiducial markers to 6DoF via photogrammetry and perform semi-active speed constrained resection, was combined with a head mounted display, to provide a video-see-through augmented reality system. The accuracy of this system was investigated by probing 180 points upon a 110x110x50 mm known geometry and performing controlled resection upon a 60x60x15 mm bone phantom guided by an overlaid augmented resection guide that updated in real-time.
The system produced an RMS probing accuracy and precision of 0.55±0.04 and 0.10±0.01 mm, respectively. Controlled resection resulted in an absolute resection error of 0.34±0.04 mm with a general trend of over-resection of 0.10±0.07 mm.
The system was able to achieve the sub-millimetre accuracy considered necessary to successfully position unicondylar knee implants. Several refinements of the system, such as pose filtering, are expected to increase the functional volume over which this accuracy is obtained. The presented system improves upon several objections to existing commercial CAOS UKA systems, and shows great potential both within surgery itself and its training. Furthermore, it is suggested the system could be readily extended to additional orthopaedic procedures requiring accurate and intuitive guidance.

19. 3D PRINTING OF ORTHOPAEDIC CLINICAL IMAGES TO AID COMPLEX SURGICAL PLANNING: A PROOF OF CONCEPT STUDY

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3D imaging is commonly employed in the surgical planning and management of bony deformity. The advent of desktop 3D printing now allows rapid in-house production of specific anatomical models to facilitate surgical planning. The aim of this pilot study was to evaluate the feasibility of creating 3D printed models in a university hospital setting.

For requested cases of interest, CT DICOM images on the local NHS Picture Archive System were anonymised and transferred. Images were then segmented into 3D models of the bones, cleaned to remove artefacts, and orientated for printing with preservation of the regions of interest. The models were printed in polylactic acid (PLA), a biodegradable thermoplastic, on the CubeX Duo 3D printer.

PLA models were produced for 4 clinical cases; a complex forearm deformity as a result of malunited childhood fracture, a pelvic discontinuity with severe acetabular deficiency following explantation of an infected total hip replacement, a chronically dislocated radial head causing complex elbow deformity as a result of a severe skeletal dysplasia, and a preoperative model of a deficient proximal tibia as a result of a severe tibia fracture. The models materially influenced clinical decision making, surgical intervention planning and required equipment. In the case of forearm an articulating model was constructed allowing the site of impingement between radius and ulnar to be identified, an osteotomy was practiced on multiple models allowing elimination of the block to supination. This has not previously been described in literature. The acetabulum model allowed pre-contouring of a posterior column plate which was then sterilised and eliminated a time consuming intraoperative step.

While once specialist and expensive, in house 3D printing is now economically viable and a helpful tool in the management of complex patients.

STRYKER MODELLING AND ANALYTICS SYSTEM: FUTURE IMPLANT DESIGN
ANDREAS PETERSIK, R&D VIRTUAL ENGINEERING
STRYKERS ADDITIVE MANUFACTURING: MAKING THE COMPLEX POSSIBLE
GEAROID WALSH, SENIOR ENGINEER, AO ADDITIVE.
Late Afternoon Breakout Session: Bridie Library

BREAKOUT SESSION WITH STRYKER EXPERTS
ANDREAS PETERSIK & GEAROID WALSH WILL BE AVAILABLE WITH OTHER STYRKER STAFF AND SAWBONES WORKSHOPS IN THE BRIDIE LIBRARY TO ANSWER ANY TECHNICAL QUESTIONS FOLLOWING THE PRECISION ENGINEERING SESSION.

LATE - AFTERNOON SESSION: DEBATES CHAMBER
INFECTION IN ORTHOPAEDICS

CHAIRING: PROF G. RAMAGE & MR D. HANSOM

20. **A MURINE MODEL OF SEPTIC ARTHRITIS DEMONSTRATES THAT INFECTION WITH AN ALPHA TOXIN PRODUCING *STRAIN OF S. AUREUS* LEADS TO SIGNIFICANTLY ELEVATED LEVELS OF CHONDROCYTE DEATH WITHIN 48 HOURS OF INFECTION WHEN COMPARED TO INFECTION WITH AN ALPHA-TOXIN DEFICIENT MUTANT STRAIN.**

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Chondrocytes are essential to the maintenance of articular cartilage and it is thought that chondrocyte death occurs early in septic arthritis. Understanding the causes of chondrocyte death will allow the development of chondroprotective strategies to improve long-term outcomes following septic arthritis.

We utilised a murine model of septic arthritis using intra-articular injection of 10µL of *a 10^7 concentration of S. aureus* suspended in PBS. Seventy-five adult male C57/Bl6 mice were randomised to receive injection of either *S. aurues* 8325-4 (a wild-type of *S. aurues* capable of alpha toxin production), DU1090 (an isogenic mutant of 8325-4 that is identical to 8325-4 other than being incapable of producing alpha toxin) or a PBS control. Establishment of septic arthritis was confirmed through gait changes (5 mice/group), limb swelling and histological changes (10 mice/group). 10 animals from each group were sacrificed at 48 hours and the injected knee joints were dissected before being stained with CFMDA (labelling live chondrocytes green) and PI (labelling dead chondrocytes red). The samples were imaged using a confocal laser scanning microscope and the percentage of chondrocyte death was calculated.

Mice injected with *S. aureus* 8325-4 or DU1090 developed septic arthritis with evidence of weight loss, limb swelling and gait changes whereas these were absent in the control group. There was a significantly higher level of chondrocyte death in the group infected with 8325-4 (2.7% chondrocyte viability) when compared to DU1090 (73.9% chondrocyte viability) and PBS injected mice (95% chondrocyte viability). One-Way ANOVA revealed that the difference between each group was statistically different ($p < 0.05$).

Alpha toxin is the major damaging toxin in *S. aurues* septic arthritis. Any adverse effect of the immune system is negligible in comparison. Development of treatments counteracting the effect of alpha toxin is required.

21. NanoCaRRIER delivery of bioactive matrices and antimicrobials for hard tissue repair

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Demineralised dentine matrix (DDM) contains a myriad of growth factors and matrix proteoglycans, the bioactivity of which can utilised in dental restorations and bone augmentations. This study aimed to develop a novel antimicrobial, bioactive dental cement to promote reparative dentinogenesis and prevent infections, improving the longevity of current dental restorations.

Nanocarriers containing DDM (extracted from non-carious dentine; 1-100 µg/mL), and triclosan (300 µg/mL) were made. Human dental pulp stem cells (hDPSCs) were treated with DDM nanocarriers (10 ng/mL-100 µg/mL) for 3, 9, 21 and 35 days. Cell proliferation and viability were assessed by cell counts, Caspase-Glo 3/7 (Promega) and MTT assays. qRT-PCR was used to examine the expression of osteogenic markers runx2 and osteocalcin at days 3, 9 and 21. A transwell chemotaxis/ migration assay was used to assess the ability of DDM nanoparticles to recruit hDPSC progenitors. Triclosan nanocarriers were tested using growth curves and zones of inhibitions for *S. Anginosus* and *E. Faecalis*. SEM and biomechanical testing was carried out on Vitremer (Henry Schein) dental cements containing loaded and empty nanocarriers.

DDM nanocarriers were able to significantly recruit hDPSCs and induce the expression of osteogenic markers in hDPSCs after 9 days. DDM Nanocarriers had no effect on cell proliferation or survival. Triclosan nanocarriers were able to inhibit the growth of *S. Anginosus* and *E. Faecalis.* Nanocarriers had limited effect on the biomechanical integrity of Vitremer cements.

Nanocarriers successfully delivered DDM to hDPSC, promoting their *in vitro* recruitment and osteogenic differentiation, and triclosan to endodontic bacteria inhibiting their growth. The nanocarriers were incorporated into cements with minimal physical artefacts, therefore a novel antimicrobial, bioactive dental cement was produced, which could be a useful tool for dental tissue engineering.

22. DEVELOPMENT OF 405 nm HINS-LIGHT TECHNOLOGY FOR DECONTAMINATION APPLICATIONS IN ARTHROPLASTY SURGERY

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Healthcare associated infections (HAI) pose a major threat to patients admitted to hospitals, and infection rates following orthopaedic arthroplasty surgery are as high as 4%, while the infection rates are even higher after revision surgery. 405 nm High-Intensity Narrow Spectrum (HINS) light has been proven to reduce environmental contamination in hospital isolation rooms, and there is potential to develop this technology for application in orthopaedic surgery.

Cultured rat osteoblasts were exposed to 405 nm light to investigate if bactericidal doses of light could be used safely in the presence of mammalian cells. Cell viability was measured by MTT reduction and microscopy techniques, function by alkaline phosphatase activity, and proliferation by the BrdU assay. Exposures of up to a dose of 36 J/cm^2 had no significant effect on osteoblast cell viability, whilst exposure of a variety of clinically relevant bacteria, to 36 J/cm^2 resulted in up to 100% kill. Exposure to a higher dose of 54 J/cm^2 significantly affected the osteoblast cell viability, indicating dose dependency.

Work also demonstrated that 405 nm light exposure induces reactive oxygen species (ROS) production in both mammalian and bacterial cells, as shown by fluorescence generated from 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate dye. The mammalian cells were significantly protected from dying at 54 J/cm^2 by catalase, which detoxifies H_2O_2. Bacterial cells were significantly protected by sodium pyruvate (H_2O_2 scavenger) and by a combination of free radical scavengers (sodium pyruvate, dimethyl thiourea ($^{\cdot}OH$ scavenger), catalase) at 162 and 324 J/cm^2. Thus the cytotoxic mechanism of 405 nm light in mammalian cells and bacteria is likely oxidative stress involving predominantly H_2O_2 generation, with other ROS contributing to the damage.

Additional work describing the potential for incorporation of this antimicrobial light within operating theatre lighting systems will also be discussed, and this, coupled with the cell viability and cytotoxicity results, suggests that 405 nm light could have great potential for continual patient safe decontamination during orthopaedic replacement surgeries and thereby reduce the incidence of infections.

23. **THE EFFECTS OF NANOPATTERN SURFACE TECHNOLOGY ON ORTHOPAEDIC JOINT REPLACEMENT INFECTION.**

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One of the most common bacteria in orthopaedic prosthetic infections is Staphylococcus Aureus. Infection causes implant failure due to biofilm production. Biofilms are produced by bacteria once they have adhered to a surface.

Nanotopography has major effects on cell behaviour. Our research focuses on bacterial adhesion on nanofabricated materials. We hypothesise that surface nanotopography impacts the differential ability of staphylococci species to adhere via altered metabolomics and may reduce orthopaedic implant infection rate.

Bacteria were grown and growth conditions optimised. Polystyrene and titanium (Ti) nanosurfaces were studied. The polystyrene surfaces had different nanopit arrays, while the Ti surfaces expressed different nanowire structures. Adhesion analysis was performed using fluorescence imaging, quantitative PCR and bacterial percentage coverage calculations. Further substitution with 'heavy' labelled glucose into growth medium allowed for bacterial metabolomic analysis and identification of any up-regulated metabolites and pathways.

Our data demonstrates reduced bacterial adhesion on specific nanopit polystyrene arrays, while nanowired titanium showed increased bacterial adhesion following qPCR ($P<0.05$) and percentage coverage calculations ($P<0.001$). Further metabolomic analysis identified significantly increased intensity counts of specific metabolites (Pyruvate, Aspartate, Alanine and Carbamoyl aspartate).

Our study shows that by altering nanotopography, bacterial adhesion and therefore biofilm formation can be affected. Specific nanopatterned surfaces may reduce implant infection associated morbidity and mortality. The identification of metabolic pathways involved in adhesion may allow for a targeted approach to biofilm eradication in S. aureus. This is of significant benefit to both the patient and the surgeon, and may well extend far beyond the realms of orthopaedics.

24. OSTEOGENIC AND BACTERICIDAL SURFACES FROM HYDROTHERMAL TITANIA NANOWIRES ON TITANIUM SUBSTRATES

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Nanotopographical cues on Ti surfaces have been shown to elicit different cell responses such as differentiation and selective growth. Bone remodelling is a continuous process requiring specific cues for optimal bone growth and implant fixation. In addition, the prevention of biofilm formation on surgical implants is a major challenge. We have identified nanopatterns on Ti surfaces that would be optimal for both bone remodelling and for reducing risk of bacterial infection. We used primary human osteoblast/osteoclast co-cultures and seeded them on flat Ti and three Ti nanosurfaces with increasing degrees of roughness, manufactured using anodisation under alkaline conditions (for 2, 2.5 and 3 hours). Cell growth and behaviour was assessed by scanning electron microscopy (SEM), immunofluorescence microscopy, histochemistry and quantitative RT-PCR methods. Bacterial growth on the nanowire surfaces was also assessed by confocal microscopy and SEM. From the three surfaces tested, the 2 h nanowire surface supported osteoblast and, to a lesser extent, osteoclast growth and differentiation. Bacterial viability was significantly reduced on the 2h surface. Hence the 2 h surface provided optimal bone remodelling conditions while reducing infection risk, making it a favourable candidate for future implant surfaces. This work was funded by EPSRC grant EP/K034898/1.

25. HOW DOES THE LEVEL OF BONE MINERALISATION AFFECT THE DEPTHS PROBED BY SPATIALLY OFFSET RAMAN SPECTROSCOPY?

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Development of more effective diagnostic and therapeutic solutions is vital to tackling the growing challenge of bone diseases and disorders in aging societies. Spatially offset Raman spectroscopy (SORS) enables the chemical specificity of conventional Raman spectroscopy to be combined with sub-surface probing. SORS has successfully been applied to transcutaneous investigations of underlying bone and shows great potential to become an *in vivo* tool for non-invasive diagnosis of various bone conditions.

The volume within the complex hierarchical bone tissue probed by SORS depends on the specimen's optical properties. Understanding the actual sampling depth is important to correctly assign detected chemical changes to specific areas in the bone. This study explores the hypothesis that the effective Raman signal recovery from certain depths requires different spatial offsets depending on the bone mineralisation.

SORS depth investigations were conducted on three bones with significantly different mineralisation levels. Thin slices (0.6 - 1.0 mm thickness) were cut from deer antler, horse metacarpal and whale tympanic bulla and stacked together (4 - 7 layers; 4.1 - 4.7 mm total thickness). A 0.38 mm thin slice of polytetrafluoroethylene (PTFE) served as reference sample and was inserted in between the layers of stacked bone slices. Raman spectra were acquired at 30 s using 830 nm excitation.

A quantitative relation between the SORS offset and the primarily interrogated depth inside the bone was established. Maximum accessible depths at small offset strongly depend on the mineralisation level. Using large spatial offsets of 7 - 9 mm PTFE signal recovery depths of 4.4 - 4.6 mm through cortical bone can be realized with only minor dependence on the bone mineralisation.

These findings highlight the potential of SORS for medical diagnostics by enabling the non-invasive detection of bone conditions characterised by chemical alterations several millimetres inside compact bone tissue (e.g. infections, tumours, etc.).

PRESIDENTIAL PRIZE LECTURE: PROF J. KENWRIGHT
CHAIRING: PROF G. BLUNN

TUESDAY 6TH SEPTEMBER
MORNING SESSION: DEBATES CHAMBER
BIOMECHANICS SESSION

CHAIRING: PROF E. TANNER & MR F. MAHMOOD

26. STRAIN FIELD MEASUREMENTS APPLIED TO ARTICULAR CARTILAGE USING DIGITAL IMAGE CORRELATION.

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Osteoarthritis is one of the most common musculoskeletal diseases. It involves degeneration and loss of articular cartilage, leading to a painful bone on bone articulation during movement. Numerical FEA models exist to predict the mechanical behaviour of degenerated cartilage. One of the limitations of these models arises from the poor validation that can be attained with traditional experimental data. This typically relies on comparison with global mechanical quantities such as

total tissue strain, which mask the individual contributions originating from the different layers. In order to improve on this, an experimental method was developed to visualise the through-thickness behaviour of articular cartilage.

Four experiments were performed on hemi-cylindrical cartilage plugs, harvested from a porcine femoral head, and immersed in a fluid solution. An Indian ink speckle pattern was applied to the flat surface of each hemi-cylinder. The specimens were equilibrated in 2.5M NaCl solution, transferred to a custom designed testing rig, and a reference image of the tissue cross-section was taken.

The solution concentration was then decreased to 0.15M and, predictably, the tissue thickness changed. Images of the tissue cross section were taken every 60s for the duration of the experiment (3600s). All images were analysed using a DIC algorithm (Ncorr open-source 2D digital image correlation matlab program), and documented the strain changes through the tissue thickness as a function of time. The measured total strain in the tissue was consistent with that reported by Lai et al. (1991). However the present technique allows to quantify the strain contribution from any of the tissue layers or sublayer. This poses a significant advantage over traditional methods as resulting information can further the understanding of the factors contributing to the mechanical behaviour of the tissue and provides an ideal platform for validating more and more refined models of tissue behaviour.

27. A NEW LANDMARK FOR MEASURING TIBIAL ROTATION AFTER TOTAL KNEE REPLACEMENT

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Since the publication by Berger in 1993, many total knee replacements (TKR) have been measured using his technique to assess component rotation. Whereas the femoral landmarks have been showed to be accurate and precise, the use of the tibial tuberosity to ascertain the true tibial orientation is more controversial. The goal of this study was to identify a new anatomical landmark to measure tibial component rotation.

211 CTs performed after TKR were reviewed. The authors noticed that the lateral cortex of the tibia below the tibial plateau component was flat over a depth of approximately 10mm. A protocol to measure tibial rotation in relation to this landmark was developed: the slice below the tibial plateau was identified; a primary line was drawn over the straight lateral cortex of the tibia; a perpendicular to this line defined the reference axis (A); the posterior tibial component axis was drawn (B); the angle between A and B was measured with internal rotation being negative and external positive. Two independent observers measured 31 CTs twice each and Intraclass Correlation Coefficients (ICC) were calculated for intra- and inter-observer error. The 211CTs were measured according to Berger's and this protocol.

Intra-observer ICCs were 0.812 for Observer1 and 0.806 for Observer2. The inter-observer ICCs were 0.699 for Reading1 and 0.752 for Reading2. The Berger protocol mean tibial rotation was 9.7°±5.5° (-29.0° to 5.2°) and for the new landmark 0°±5.4° (-18.6° to 14°).

This new tibial landmark appeared easy to identify and intra- and inter-observer errors were acceptable. The fact that the mean tibial rotation was 0° makes this landmark attractive. A consistent easily identified landmark for tibial rotation may allow for improvement in component rotation and the diagnosis of dissatisfaction after TKR. Further studies are under way to confirm the relevance of this landmark.

28. Identification of movement strategies during the sit-to-walk movement in patients with knee osteoarthritis.

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Patients with osteoarthritis (OA) of the knee commonly alter their movement to compensate for deficiencies. This study presents a new numerical procedure for classifying sit-to-walk (STW) movement strategies.

Ten control and twelve OA participants performed the STW task in a motion capture laboratory. A full body biomechanical model was used. Participants were instructed to sit in a comfortable self-selected position on a stool height adjusted to 100% of their knee height and then stand and pick up an object from a table in front of them. Three matrices were constructed defining the progression of the torso, feet and hands in the sagittal plane along with a fourth expressing the location of the hands relative to the knees. Hierarchical clustering (HC) was used to identify different strategies. Trials were also classified as to whether the left (L) and right (R) extremities used a matching strategy (bilateral) or not (asymmetrical). Fisher's exact test was used to compare this between groups.

Clustering of the torso matrix dichotomised the trials in two major clusters; subjects leaning forward (LF) or not. The feet and hands matrices revealed sliding the foot backward (FB) and moving an arm forward (AF) strategies respectively. Trials not belonging in the AF cluster were submitted to the last HC of the fourth matrix exposing three additional strategies, the arm pushing through chair (PC), arm pushing through knee (PK) and arm not used (NA). The control participants used the LF+FBR+PK combination most frequently whereas the OA participants used the AFR+PCL. OA patients used significantly more asymmetrical arm strategies, p=0.034.

The results demonstrated that control and OA participants favour different STW strategies. The OA patients asymmetrical arm behaviour possibly indicates compensating for weakness of the affected leg. These strategy definitions may be useful to assess post-operative outcomes and rehabilitation progress.

29. THE EFFECT OF K TAPE ON VASTUS MEDIALIS OBLIQUE AND RECTUS FEMORIS MUSCLE ACTIVITY AND CRTICAL KNEE FLEXION ANGLE AT WHICH DYNAMIC VALGUS OCCURS DURING A SINGLE- LEG SQUAT.

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Patellofemoral pain syndrome (PFPS) is a common knee disorder in active individuals. Movement dysfunction of valgus positioning at the knee during weight-bearing is frequently seen in PFPS. A single-leg squat (SLS) is a test commonly used in physiotherapy to assess for movement dysfunction. Kinesio-Tape (KT) is gaining in popularity in treating PFPS and claims to alter muscle recruitment and motor control, however evidence is weak. Objective: To evaluate the effect of KT applied to the quadriceps on muscle activity with electromyography (EMG) of the rectus femoris, vastus lateralis and vastus medialis oblique and motor control via the frontal plane projection angle (FPPA) using 2-dimensional video analysis.

A convenience sample of healthy females were recruited and performed 5 single-leg squats with and without KT. EMG of the quadriceps was recorded and dynamic valgus assessed via the FPPA using Dartfish video analysis software. Eccentric and concentric EMG data was recorded and the FPPA measured in single-leg stance and the depth of the squat. Institutional ethical approval was obtained for the study.

16 active females were assessed (mean age 28.94 $\pm$6.58 years). Wilcoxon signed-rank tests found no significant change in eccentric or concentric EMG of the quadriceps (%MVC) with KT compared to without (p values 0.35-0.86). Paired-sample t-tests found no significant difference in FPPA between conditions in single-leg stance (p=1.00) or the depth of the squat (p=0.871).

KT did not affect EMG activity of the quadriceps or the FPPA in a SLS when applied to the quadriceps of healthy females, questioning proposed effects of KT on normal muscle tissue. Further research is required into the efficacy of using KT in physiotherapy.

30. MEASURING CORTICAL BONE STIFFNESS USING MICRO-INDENTATION

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The increase in revision joint replacement surgery and fractures of bone around orthopaedic implants may be partly addressed by keeping bone healthy around orthopaedic implants by inserting implants with mechanical properties closer to the patient's bone properties. We do not currently have an accurate way of calculating a patient's bone mechanical properties. We are therefore investigating whether microindentation can accurately calculate bone stiffness.

We received ethical approval to retrieve femoral heads and necks from patients undergoing hip replacement surgery for research. Cortical bone from the medial calcar region of the femoral neck was cut into 3x3x6mm cuboid specimens. Micro-indentation testing was performed in the direction of loading of the bone using a MicroMaterials indenter. The samples were kept hydrated and were not fixed or polished. From the unloading curve after indentation, the elastic modulus was calculated, using the Oliver- Pharr method. To assess which microindentation machine settings most precisely calculate the elastic modulus we varied the loading and unloading rates, load and indenter tip shape.

The most precise results were obtained by using a spherical indenter tip (rather than Berkovich tip), high load (10N), a loading rate of 100 mN/s and unloading rate of 300 mN/s with a pause of 60 seconds at maximum load and multiple load cycles with constant loads. Using these settings the mean elastic modulus over 12 cycles of testing was 13.0 GPa (+/- 2.47).

By using a spherical indenter tip and fast unloading it was possible to get precise apparent modulus values. By unloading as fast as possible the effects of bone viscoelastic properties are minimised. By using a spherical indenter tip, plastic deformation at the tip is minimised (compared to the Berkovich tip). We are performing further standard compression tests on the samples to verify the accuracy of the indentation tests.

MORNING SESSION: DEBATES CHAMBER

ACADEMIC OSTEOARTHRITIS SESSION

CHAIRING: PROF G. BLUNN & DR C. GOODYEAR

31. PATIENT MUSCLE SATELLITE CELL CONTENT IS A POTENTIAL BIOMARKER FOR PHYSCIAL RECOVERY AND CLINICAL OUTCOME FOLLOWING TOTAL KNEE ARTHROPLASTY

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Physical outcome following total knee arthroplasty is variable. Satellite cells are undifferentiated myogenic precursors considered to be muscle stem cells. We hypothesised that; the recovery of muscle strength and physical function following knee arthroplasty would be influenced by the underlying number of muscle satellite cells.

16 patients provided a distal quadriceps muscle biopsy at time of surgery. Satellite cells were identified with a primary mouse antibody for Pax7 - a cytoplasmic protein marker, and the myonuclei with DAPI. Positive cells were identified on the basis of immunofluorescent staining in association with nuclear material, and confirmed by position under the basal lamina. Patient function was assessed using a validated physical assessment protocol, the Aggregated Locomotor Function (ALF) score, muscle strength assessed using the leg extensor power-rig, and clinical outcome assessed with the Oxford Knee Score (OKS) pre-operatively and at 1 year post operatively.

Muscle satellite cell content varied amongst the patient group (Positive Staining Index 3.1 to 11.4). Satellite cell content at time of surgery correlated with change in outcomes between pre-operative and 1 year assessments in all assessed parameters (ALF, r = 0.31; muscle power, r = 49; OKS, r = 0.33). Regression analysis employing a forward stepwise selection technique employed satellite cell volume in models of pre-operative to 1 year change for all outcome parameters. Physical function (satellite cell content, patient age and pre-operative ALF score) adjusted R2 = 0.92; Muscle power (pre-operative power and satellite cell content) adjusted R2 = 0.38; Clinical outcome (pre-operative OKS and satellite cell content) adjusted R2 = 0.28.

Muscle satellite cell content influences recovery of muscle power and physical function following total knee arthroplasty. Importantly it is also associated with change in clinical scores; suggesting it to be a biomarker for patient outcomes.

32. TRIM32 DEFICIENCY IS ASSOCIATED WITH INCREASED CARTILAGE DEGRADATION AND ALTERED CHONDROCYTE PHENOTYPE IN HUMAN AND MURINE ARTICULAR TISSUE *EX VIVO*

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TRIM32 is a candidate gene at the 9q33.1 genetic susceptibility locus for hip osteoarthritis (OA). Increased cartilage degradation typical of OA has previously been demonstrated in *Trim32* knockout mice.

Our aim is to investigate the role of TRIM32 in human and murine articular tissue.

TRIM32 expression in human articular cartilage was examined by immunostaining. TRIM32 expression was compared in femoral head chondrocytes from patients with and without primary hip OA (n=6/group) and examined by Western blotting. Aggrecanolysis by femoral head explants from *Trim32* knockout (T32KO) and wild-type (WT) mice was compared following stimulation with IL1α or retinoic acid (RA) and was assessed by DMMB assay (n=4/group). Expression of chondrocyte phenotype markers was measured by qPCR and compared between articular chondrocytes from WT and T32KO mice following catabolic (IL1α/TNFα) or anabolic (Oncostatin-M (OSM)/IGF1) stimulation.

TRIM32 expression was demonstrated in human articular cartilage; TRIM32 expression by chondrocytes was reduced in patients with hip OA (p=0.03). Greater aggrecanolysis occurred in cartilage explants from T32KO mice after treatment with no stimulation (p=0.03), IL1α (p=0.02), and RA (p=0.001). Unstimulated T32KO chondrocytes expressed reduced *Col2a1* ($p=8.53\times10^{-5}$), and *Sox9* ($p=2.35\times10^{-6}$). Upon IL1α treatment, T32KO chondrocytes expressed increased *Col10a1* (p=0.0003). Upon anabolic stimulation, T32KO chondrocytes expressed increased *Col2a1* (OSM: p=0.001; IGF: p=0.001), and reduced *Sox9* (OSM: p=0.0002; IGF: p=0.0006).

These results indicate that altered TRIM32 expression in human articular tissue is associated with OA, and that *Trim32* knockout results in increased cartilage degradation in murine femoral head explants. Predisposition to cartilage degeneration with reduced *Trim32* expression may involve increased chondrocyte hypertrophy upon catabolic cytokine stimulation and dysregulation of *Col2a1* and *Sox9* expression upon anabolic stimulation.

33. THE EFFECTS OF CHRONIC COBALT AND CHROMIUM EXPOSURE AFTER METAL-ON-METAL HIP RESURFACING ON DNA METHYLATION: AN EPIGENOME-WIDE ASSOCIATION STUDY

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Systemic concentrations of metal ions (cobalt and chromium) are persistently elevated in patients with metal-on-metal hip resurfacing (MOMHR) compared to conventional total hip arthroplasty (THA). Several studies by us and others have described the detrimental effects of metal exposure on survival and function of various cell types *in-vitro,* but the mechanisms for these effects remain unclear. Epigenetic modifications following chronic metal exposure is a possible mechanism that could mediate these effects. Here we test the methylation status in genomic DNA from MOMHR ("cases") and THA ("controls") patient-groups, and its correlation with circulating metal levels.

The cohort consisted of 34 patients with a well-functioning MOMHR at a median follow-up of 9.75 years. These were individually matched for gender, age and time-since-surgery to a non-exposure group consisting of patients with THA. Genomic DNA was isolated from blood samples and cell composition estimated using the 'estimateCellCounts' function in 'minfi R-package'. Methylation was assessed using the Illumina 450k BeadChip array analysing 426,225 probes. Logit model was fitted at each probe with case/control status as independent variable and covariates of gender, age, time-since-surgery, smoking, non-arthroplasty metal exposure, and cell composition. DNA methylation age was assessed using an online calculator (https://dnamage.genetics.ucla.edu/) and comparisons made between cases and controls, and correlated with circulating metal levels.

Cell distributions did not differ between the cases and controls (Wilcoxon test $p>0.17$) with no probe having an association at 5% FDR. Circulating metal levels and LVEDD also had no association with any probe at 5% FDR. There was no preferential age acceleration between cases and controls (Wilcox $p>0.7$), and it had no correlation with plasma-chromium or blood-cobalt levels ($p>0.9$).

In summary, large methylation changes following MOMHR seem to be absent, compared to THA. Future research with larger samples will be needed to clarify the presence and extent of small methylation changes.

34. COMPARISON OF THE CYTOTOXTIC EFFECTS OF CLINICALLY-RELEVANT COBALT-CHROMIUM WEAR PARTICLES AND COMMERCIAL COMPOSITE CERAMIC PARTICLES

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Wear particles produced by alumina ceramic-on-ceramic (CoC) bearings cause a minimal immunological response with low cytotoxicity and inflammatory potential[1, 2]. However, more comprehensive immunological studies are yet to be completed for the composite CoC (zirconia-toughened, platelet reinforced alumina) hip replacements due to difficulties in isolating the very low volume of clinically relevant wear debris generated by such materials *in vitro*. The aim of this study was to compare the cytotoxic effects of clinically relevant cobalt chromium (CoCr) nano-particles with commercial composite ceramic particles.

Composite ceramic particles (commercial BIOLOX® delta powder) were obtained from CeramTec, Germany and clinically relevant CoCr wear particles were generated using a six station pin-on-plate wear simulator. L929 fibroblast cells were cultured with 50μm^3 of CoCr wear debris or composite ceramic particles at low to high volumes ranging from 500μm^3-0.5μm^3 per cell and the cyctotoxic effects of the particles were assessed over a period of 6 days using the ATP-Lite™ cell viability assay.

The composite ceramic particles were bimodal in size (0.1-2µm & 30-100nm) and showed mild cytotoxic effects when compared with equivalent particle volumes (50μm^3) of clinically relevant CoCr nano-particles (10-120nm). The CoCr nano-particles had significant cytotoxic effects from day 1, whereas the composite ceramic particles only showed cytotoxic effects at particle concentrations of 50 and 500μm^3 after 6 days. The increased cytotoxicity of the clinically relevant CoCr nano-particles may have been attributed to the release of Co and Cr ions.

This study demonstrated the potential cytotoxic effects of model ceramic particles at very high volume concentrations, but it is unlikely that such high particle volumes will be experienced routinely *in vivo* in such low wearing bearing materials. Future work will investigate the longer-term effects on genotoxicity and oxidative stress of low volumes of clinically-relevant generated BIOLOX® *delta* ceramic wear particles.

References

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35. EFFECT OF CIRCULATING METAL IONS ON SURVIVAL AND FUNCTION OF OSTEOCLASTS FROM PATIENTS FOLLOWING HIP RESURFACING

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We have previously observed an increase in total bone mineral density and reduced bone turnover (TRAP5b and osteocalcin) in patients with well-functioning metal-on-metal hip resurfacing (MOMHR). Here, we provide data to support the hypothesis that osteoclast differentiation and function is altered in this patient population, and that this effect is transferrable through their serum.

Patients with well-functioning MOMHR (cases, n=18) at a median follow-up of 8 years were individually matched for gender, age and time-since-surgery to a low-exposure group consisting of patients with THA (controls, n=18). The monocyte fraction of patient peripheral blood was isolated and differentiated into osteoclasts on dentine wafers using RANKL and M-CSF supplemented media (osteoclastogenic media, OM). Cultures were monitored for the onset of resorption, at which point the cells were treated with OM, autologous serum or serum from matched MOMHR/THA donors, all supplemented with RANKL and M-CSF. At the end of the culture, cells were TRAP-stained and quantified using CellD Software Package, Olympus.

When cells were differentiated in standard osteoclastogenic media, the resorbing ability of osteoclasts derived from MOMHR patients was reduced 22%($p<0.0079$) compared to THA. The resorbing ability of osteoclasts generated from MOMHR patients and differentiated in autologous serum was reduced 33%($p<0.0001$), whilst matched THA serum caused a smaller reduction of 14%($p<0.01$). When cells derived from THA patients were differentiated in autologous serum, the resorbing ability of osteoclasts was similarly reduced by 35%($p<0.0001$), whilst the matched MOMHR serum also caused a reduction of 21%($p<0.0001$).

This data suggests that prior exposure to higher circulating Co and Cr in patients with MOMHR reduces osteoclastogenesis, and that the detrimental effect on the functionality of mature osteoclasts is transferable through the serum. This has implications for systemic bone health of patients with MOMHR or modular taper junctions.

36. OSTEOCLAST PREVALENCE WITHIN BONE MARROW LESIONS AND SYNOVITIS SEVERITY AND THEIR ASSOCIATION WITH NEUROPATHIC PAIN AND CENTRAL SENSITIZATION IN KNEE OSTEOARTHRITIS

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A subgroup of patients that undergo TKR surgery have evidence of neuropathic pain and central sensitization that may predispose to severe postoperative pain. This study assesses the correlation of MRI detected bone marrow lesions (BMLs) and synovitis with markers of neuropathic pain and central sensitization in patients undergoing TKR surgery and healthy volunteers.

31 patients awaiting TKR and 5 healthy volunteers were recruited. Each subject underwent a 3-T knee MRI scan that was graded for BMLs (0-45) and synovitis (0-3) using subsets of the MRI Osteoarthritis Knee Score (MOAKS). All subjects were asked to complete the PainDetect questionnaire to identify nociceptive pain (< 13), unclear pain (13-18) and neuropathic pain (>18). Correlation between BMLs and PainDetect score was the primary outcome measure. Secondary outcomes included the correlation of synovitis to PainDetect and temporal summation (TS) a measure of central sensitization to the PainDetect score. TS was determined using a monofilament to evoke pain. Pilot histological analysis of the prevalence of osteoclasts ($TRAP^+$) within BMLs versus normal subchondral bone was performed, implying a role in BML pathology.

Increasing BML MOAKS score correlated with neuropathic pain (painDetect), $r_s = 0.38$, p=0.013 (one-tailed). There was a positive correlation between synovitis and PainDetect score, $\tau = 0.23$, p= 0.031 (one-tailed). TS was greater in the neuropathic pain than in nociceptive pain patients, U = 18.0, p=0.003 (one-tailed). TRAP staining identified more osteoclasts within BMLs than contralateral condyle lesion free subchondral bone, z = -2.232, p = 0.026 (Wilcoxon Signed Rank Test, one-tailed).

BMLs and synovitis are more prevalent in neuropathic pain and central sensitization in knee OA. Higher osteoclast prevalence was seen within BMLs which may help explain the association with BMLs and pain in OA.

Mid - Morning Session: Debates Chamber

BLASTED BONES AND WORN OUT JOINTS

CHAIRING: MR R.M.D. MEEK & MR P.S. YOUNG

KEYNOTE: BLASTED BONES! SKELETAL FAILURE AFTER EXPLOSIONS
PROFESSOR JON CLASPER

Professor in Bioengineering, Imperial College London, Clinical Lead, The Royal British Legion Centre for Blast Injury Studies.
As a Defence Professor in Trauma & Orthopaedics he is responsible for the orthopaedic research focus of the British military, and a founding member of the Imperial Blast group. Professor Clasper will talk to us about his experiences in dealing with blast trauma and its effect on the skeleton.

37. HOW DOES IMPLANT TYPE, HEAD SIZE AND AVN LESION SIZE AFFECT THE LIKELIHOOD OF FEMORAL HEAD COLLAPSE FOLLOWING HIP FIXATION?

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Femoral head collapse due to avascular necrosis (AVN) is a relatively rare occurrence following intertrochanteric fractures; however, with over thirty-thousand intertrochanteric fractures per year in England and Wales alone, and an incidence of up to 1.16%, it is still significant. Often patients are treated with a hip fixation device, such as a sliding hip screw or X-Bolt. This study aimed to investigate the influence of three factors on the likelihood of head collapse: (1) implant type; (2) the size of the femoral head; and (3) the size of the AVN lesion.

Finite element (FE) models of an intact femur, and femurs implanted with two common hip fixation designs, the Compression Hip Screw (Smith & Nephew) and the X-Bolt (X-Bolt Orthopaedics), were developed. Experimental validation of the FE models on 4^{th} generation Sawbones composite femurs (n=5) found the peak failure loads predicted by the implanted model was accurate to within 14%. Following validation on Sawbones, the material modulus (E) was updated to represent cancellous (E=500MPa) and cortical (E=1GPa) bone, and the influence of implant design, head size, and AVN was examined. Four head sizes were compared: mean male (48.4 mm) and female (42.2 mm) head sizes ± two standard deviations. A conical representation of an AVN lesion with a lower modulus (1MPa) was created, and four different radii were studied. The risk of head collapse was assessed from (1) the critical buckling pressure and (2) the peak failure stress.

The likelihood of head collapse was reduced by implantation of either fixation device. Smaller head sizes and greater AVN lesion size increased the risk of femoral head collapse. These results indicate the treatment of intertrochanteric fractures with a hip fixation device does not increase the risk of head collapse; however, patient factors such as small head size and AVN severity significantly increase the risk.

38. PREDICTING CHRONIC POST-OPERATIVE PAIN AFTER TKR BY PREOPERATIVE ASSESSMENT OF CENTRAL SENSITIZATION USING FUNCTIONAL BRAIN MRI AND SEMI-QUANTITATIVE MRI SCORING OF THE KNEE. A PRELIMINARY CASE-CONTROL STUDY.

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Email:

Painful OA is linked to CNS changes in pain processing. Temporal summation of pain (TSP) is a measure of one such CNS change, central sensitization. TSP is defined using a series (≥0.33Hz) of painful stimuli and is a predictor of postoperative pain, experienced by 20% of patients after total knee replacement (TKR) surgery. This

study has developed a protocol to use functional MRI to assess CNS changes in OA pain processing.

This pilot includes 3 participants with chronic knee OA pain awaiting TKR (62 ± 4.4) and 5 healthy volunteers (50 ± 13.6). 3-Tesla BOLD fMRI brain scans were recorded during short series of one second painful stimuli, applied using an automated inflatable cuff to the calf muscle of the leg with the affected knee or left side in healthy volunteers. The pain intensity at onset and during the 10 painful stimuli were recorded using a numerical rating scale. The pattern of brain activation was averaged across noxious stimuli, and the differential activation compared the 1st vs. 10th (last) stimulus. Bone marrow lesions (BMLs), synovitis and effusion size were scored from 3-Tesla knee MRI's using MOAKS scoring.

TSP was raised in OA patients compared to control group (p=0.023). TSP brain activity in the chronic OA patients displayed higher signal within the subgenual anterior cingulate (sgACC) compared to healthy volunteers. Knee MRI identified OA patient's exhibited higher BML scores (p=0.038) and more knee effusion (p=0.018), but the lack of synovitis did not differ from control group (p=0.107).

Enhanced TSP in chronic knee OA pain may be linked with augmented responses in emotional circuitry. BMLs and effusion size appear to contribute more with pain than synovitis. These results may help understand sensitization to improve outcomes for patients with knee OA undergoing TKR surgery.

39. PATIENT FUNCTION FOLLOWING ASEPTIC REVISION TOTAL KNEE ARTHROPLASTY WITH SEMI-CONSTRAINED PROSTHESES IS THE SAME AS FOLLOWING PRIMARY KNEE ARTHROPLASTY

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Patient function is poorly characterised following revision TKA. Modern semi-constrained implants are suggested to offer high levels of function, however, data is lacking to justify this claim.

52 consecutive aseptic revision TKA procedures performed at a single centre were prospectively evaluated; all were revision of a primary implant to a Triathlon total stabilizer prosthesis. Patients were assessed pre-operatively and at 6, 26, 52 and 104 weeks post-op. Outcome assessments were the Oxford Knee Score (OKS), range of motion, pain rating scale and timed functional assessment battery. Analysis was by repeated measures ANOVA with post-hoc Tukey HSD 95% simultaneous confidence intervals as pairwise comparison. Secondary analysis compared the results of this revision cohort to previously reported primary TKA data, performed by the same surgeons, with identical outcome assessments at equivalent time points.

Mean age was 73.23 (SD 10.41) years, 57% were male. Mean time since index surgery was 9.03 (SD 5.6) years. 3 patients were lost to follow-up. All outcome parameters improved significantly over time (p <0.001). Post-hoc analysis demonstrated that all outcomes changed between pre-op, 6 week and 26 weeks post-op assessments.

No difference was seen between primary and revision cohorts in OKS (p = 0.2) or pain scores (p=0.19). Range of motion and functional performance was different between groups over the 2 year period (p=0.03), however this was due to differing pre-operative scores, post-hoc analysis showed no difference between groups at any post-operative time point.

Patients undergoing aseptic revision TKA with semi-constrained implants made substantial improvements in OKS, pain scores, knee flexion, and timed functional performance, with the outcomes achieved comparable to those of primary TKA. High levels of function can be achieved following revision knee arthroplasty, which may be important considering the changing need for, and demographics of, revision surgery.

40. DYNAMIC SEPARATION, WEAR AND DEFORMATION OF METAL-ON-POLYETHYLENE BEARINGS UNDER VARIATIONS IN COMPONENT POSITIONING

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Edge loading due to dynamic separation can occur due to variations in component positioning such as a steep cup inclination angle (rotational) or mismatch between the centres of rotation of the head and the cup (translational). The aim of this study was to determine the effect of variations in rotational and translational positioning of the cup on the magnitude of dynamic separation, wear and deformation of metal-on-polyethylene bearings.

Eighteen 36mm diameter metal-on-polyethylene hip replacements were tested on an electromechanical hip simulator. Standard gait with concentric head and cup centres were applied for cups inclined at 45° (n=3) and 65° (n=3) for two million cycles. A further two tests with translational mismatch of 4mm applied between the head and cup bearing centres for cups inclined at 45° (n=6) and 65° (n=6) were run for three million cycles. Wear was determined using a microbalance and deformation by geometric analysis. Confidence intervals of 95% were calculated for mean values, and t-tests and ANOVA were used for statistical analysis ($p<0.05$).

Under 4mm mismatch conditions, a steeper cup inclination angle of 65º resulted in larger dynamic separation (2.1±0.5mm) compared with cups inclined at 45º (0.9±0.2mm). This resulted in larger penetration at the rim under 65º (0.28±0.04mm) compared to 45º (0.10±0.09mm) cup inclination conditions ($p<0.01$). Wear rates under standard concentric conditions were 12.8±3.8 mm^3/million cycles and 15.4±5.0 mm^3/million cycles for cups inclined at 45° and 65° respectively. Higher wear rates were observed under 4mm of translational mismatch compared with standard concentric conditions at 45° (21.5±5.5 mm^3/million cycles, $p<0.01$) and 65° (23.0±5.7 mm^3/million cycles, $p<0.01$) cup inclination.

Edge loading under dynamic separation conditions due to translational mismatch resulted in increased wear and deformation of the polyethylene liner. Minimising the occurrence and severity of edge loading through optimal component positioning may reduce the clinical failure rates of polyethylene.

41. WEAR ASSESSMENT OF METAL-ON-METAL CERVICAL TOTAL DISC REPLACEMENT UNDER STANDARD ISO TESTING PROTOCOL.

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Surgical interventions for the treatment of chronic neck pain, which affects 330 million people globally [1], include fusion and cervical total disc replacement (CTDR). Most of the currently clinically available CTDRs designs include a metal-on-polymer (MoP) bearing. Numerous studies suggest that MoP CTDRs are associated with issues similar to those affecting other MoP joint replacement devices, including excessive wear and wear particle-related inflammation and osteolysis [2,3]. A device with a metal-on-metal (MoM) bearing has been investigated in the current study.

Six MoM CTDRs made from high carbon cobalt-chromium (CoCr) were tested in a six-axis spine simulator, under standard ISO testing protocol (ISO-18192-1) for a duration of 4 million cycles (MC). Foetal bovine calf serum (25%v/v), used as a lubricant, was changed every 3.3x10 cycles and saved for particle analysis. Components were taken down for measurements after each 10 cycles; surface roughness, damage modes and gravimetric wear were assessed.

The mean wear rate of the MoM CTDRs was 0.24mm/MC (SD=0.03), with the total volume of 0.98mm (SD=0.01) lost over the test duration. Throughout the test, the volumetric wear was linear; no significant bedding-in period was observed. The mean pre-test surface roughness decreased from 0.019µm (SD=0.005) to 0.012µm (SD=0.002) after 4MC of testing. Prior to testing, fine polishing marks on the bearing surfaces were observed using light microscopy. Following 4MC of testing, these polishing marks had been removed. Consistently across all components, surface discolouration and multidirectional, criss-crossing, circular wear tracks, caused by abrasive wear, were observed.

The wear results showed low wear rates exhibited by MoM CTDRs (0.24mm/MC), when compared CTDR designs incorporating metal-on-polymer bearings (0.56mm/MC) [4] as well as MoM lumbar CTDRs [5,6] (0.76mm/MC – 6.2mm/MC). These findings suggest that MoM CTDRs are more wear resistant than MoP CTDRs, however the particle characterisation and biological consequences of wear remain to be determined.

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42. IMPACT OF OBESITY ON PATIENT-REPORTED OUTCOMES FOLLOWING TOTAL KNEE ARTHROPLASTY

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Obese patients undergoing total knee arthroplasty (TKA) face increased risks of complications such as joint infection and early revision. However, the influence of obesity on measures of patient function following TKA is poorly defined.

Knee arthroplasty outcome data for procedures carried out over an eight month period was extracted from a regional database in the UK. We analysed the impact of weight categories (BMI<30, BMI=30-34.9, and BMI≥35) on the Forgotten Joint Score - 12 (FJS-12) and Oxford Knee Score (OKS). Data was available preoperatively and 12 months postoperatively. Physical and mental health was assessed with the SF-12 one year after surgery.

Data from 256 patients were available. 49.6% had a BMI<30, 27.4% had a BMI 30-34.9 and 23.1% had a BMI≥35. Mean FJS-12 results at 1-year were 48.7 points for patients with a BMI<30, 40.7 points for patients with a BMI=30-34.9 and 34.0 points for patients with a BMI≥35. Effect sizes for change from baseline to 12-month post-op were 3.0 (Cohen's d) in patients with BMI<30 and d=2.2 in patients with BMI≥35. Mean OKS results at 1 year were 36.9 (BMI<30), 33.7 (BMI=30-34.9) and 32.0 (BMI≥35) respectively. Effect sizes for change from baseline to 12-month was d=2.1 (BMI<30) and d=1.9 (BMI≥35). Differences between BMI groups with regard to post-operative change were statistically significant for the FJS-12 (p=0.038) but not for the OKS (p=0.229).

This study highlights that outcome scores may differ in their ability to capture the impact of obesity on patient function following TKA. The FJS-12 showed significant differences in outcome based on patient obesity category, whereas the OKS did not detect between group differences.

43. DAMAGE MODE ANALYSIS OF 22 AES TOTAL ANKLE REPLACEMENT EXPLANTS.

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Total ankle replacement (TAR) has a mean survivorship of 77% at 10 years which is poor compared to other types of joint arthroplasty. Osteolysis and aseptic loosening are commonly cited TAR failure modes, the mechanisms of which are unknown. Retrieval analyses of TAR devices may reveal mechanisms of failure similar or dissimilar to other joint replacements. This study investigated whether TAR explants exhibit similar damage modes to those recognised in other total joint replacements.

22 Ankle Evolution System TARs (Transystème, Nimes, France) were implanted and retrieved by the same surgeon. Mean implantation time was 7.8 yrs (5.3 to 12.1 range). Pain and/or loosening were the indications for revision. Macro photography, an Alicona Infinite microscope and the Hood/Wasielewski scale were used to classify damage modes on the polyethylene insert. Scanning electron microscopy with energy dispersive X-ray spectroscopy was used to determine the composition of third body debris and to image the fixation surface of the tibial components.

Mean damage score was 185.4 (± 40.0 SD). Damage modes common to total knee replacements were identified on both the superior and inferior insert surfaces, these included: burnishing, scratching, pitting and abrasion. Titanium particles, hydroxyapatite fragments and bone debris were embedded in the insert surfaces. Fixation surface delamination was identified by the ongrowth of tissue between the cobalt chromium substrate and titanium alloy coating.

Damage modes indicative of high levels of wear and deformation were evident. Pitting caused by third body debris was abundant and suggested fixation surface wear and failure.

44. SUBCHONDRAL BONE LOSSES DURING OSTEOARTHRITIS PROGRESSION

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Osteoarthritis (OA) affects bone cartilage and underlying bone. Mechanically, the underlying bone provides support to the healthy growth of the overlying cartilage. However, with the progress of OA, bone losses and cysts occur in the bone and these would alter the biomechanical behaviour of the joint, and further leading to bone remodelling adversely affect the overlying cartilage.

Human femoral head and femoral condyle were collected during hip or knee replacement operation due to the end stage of osteoarthritis (age 50-70), and the cartilage patches were graded and marked. A volunteer patient, with minor cartilage injury in his left knee while the right knee is intact, was used as control. Peripheral quantitative computed tomography (pQCT) was used to scan the bone and to determine the volumetric bone mineral density (vBMD) distribution.

The examination of retrieved tissue explants from osteoarthritic patients revealed that patches of cartilage were worn away from the articular surface, and patches of intact cartilage were left. The cysts, ranging from 1 to 10mm were existed in all osteoarthritic bones, and were located close to cartilage defects in the weight-bearing regions, and closely associated with the grade of cartilage defect as measured by pQCT. The bone mineral density (vBMD) distribution demonstrated that the bones around cysts had much higher vBMD than the trabecular bone away from the cysts. Compared to the subchondral bone under thicker cartilage, subchondral bone within cartilage defect has higher vBMD. This may result from the mechanical stimulation as a result of bone-bone direct contact with less protection of cartilage in cartilage defect regions.

This study showed an association between cartilage defect and subchondral bone mineral density distribution. Cysts were observed in all osteoarthritic samples and they are located close to cartilage defects in the weight-bearing regions. Cartilage defect altered the loading pattern of the joints, this leading to the bone remodelling and resultant bone structural changes as compared to the normal bone tissues.

This work was financially supported by The ARUK Proof of Concept Award (grant no: 21160).

45. The effect of tourniquet use on the distribution of local anaesthetic in adductor canal blocks for total knee replacement: A cadaveric study

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Adductor canal blocks offer an alternative to femoral nerve block for postoperative pain relief in knee arthroplasty. They may reduce the risk of quadriceps weakness, allowing earlier mobilisation of patients postoperatively. However, little is known about the effect of a tourniquet on the distribution of local anaesthetic in the limb.

Ultrasound-guided adductor canal blocks were performed on both thighs of five human cadavers. Left and right thighs of each cadaver were randomised to tourniquet or no tourniquet for one hour. Iohexol radio-opaque contrast (Omnipaque 350) was substituted for the local anaesthetic for X-Ray imaging. All limbs underwent periodic flexion and extension during this hour to simulate positioning during surgery. The cadavers were refrozen. Fiducial markers were inserted into the frozen tissue. X-rays were obtained in 4 planes (AP, lateral 45° oblique/medial oblique, lateral). University Research Ethics Approval was obtained and cadavers were all pre-consented for research, imaging and photography according to the Anatomy Act (1984).

Analysis of radiographs showed contrast distribution in all thighs to be predominantly on the medial aspect of the thighs. The contrast margins were entire and well circumscribed, strongly suggesting it was largely contained within the aponeurosis of the adductor canal. Tourniquets appeared to push the contrast into a narrower and more distal spread along the length of the thigh compared to a more diffuse spread for those without. Proximal spread towards the femoral triangle was reduced in limbs without tourniquets.

The results suggest that contrast material may remain within the adductor canal structures during adductor canal blocks. Tourniquets may cause greater distribution of contrast proximally and distally in the thigh, but this does not appear to be clinically significant. Further studies might include radio-stereo photometric analysis using the fiducial markers in the limbs and in vivo studies to show the effect of haemodynamics on distribution.

46. ROTATIONAL ALIGNMENT OF THE DISTAL FEMUR IN TOTAL KNEE ARTHROPLASTY: AN MRI ANALYSIS

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Three distal femoral axes have been described to aid in alignment of the femoral component; the Trans Epicondylar Axis (TEA), the Posterior Condylar Axis (PCA) and the Antero Posterior (AP) axis. Our aim was to identify if there was a reproducible relationship between the axes which would aid alignment of the femoral component. This is the first study compare all three distal femoral axes with each other using magnetic resonance imaging (MRI) in a Caucasian population. Our sample group represents real life patients awaiting total knee arthroplasty (TKA), as opposed non-arthritic or cadaveric knees.

We identified the relationship between these rotational axes by performing MRI scans on 89 patients awaiting TKA with patient-specific instrumentation. Measurements were taken by two observers.

Patients had a mean age of 62.5 years (range 32-91). 51 patients were female. The mean angle between the TEA and the AP axis was 92.78° with a standard deviation of 2.51° (range 88° - 99°). The mean angle between the AP axis and the PCA was 95.43° with a standard deviation of 2.75° (range 85° - 105°). The mean angle between the TEA and the PCA was 2.78° with a standard deviation of 1.91° (range 0° - 10°).

We conclude that while there is a reproducible relationship between the differing femoral axes, there is a significant range in the relationship between the femoral axes. This range may lead to greater inaccuracy than has previously been appreciated when defining the rotation of the femoral component. There is most variation between the PCA and the AP axis. The TEA's relationship with the PCA and AP appears important in defining rotation. Due to the well accepted difficulty in defining the TEA intra-operatively, there may be a role for patient-specific instrumentation in TKA surgery with pre-operative MRI.

Afternoon Session: Debates Chamber

BONE MINERALISATION & OSTEOPOROSIS

CHAIRING: DR P.M. TSIMBOURI & MR D. RUSSELL

KEYNOTE: MANIPULATING OSSIFICATION THROUGH CHEMISTRY
LIAM GROVER

Director of Research, School of Chemical Engineering, University of Birmingham.

47. THE EFFECTS OF MITOCHONDRIAL DYSFUNCTION ON OSTEOBLAST FUNCTION IN THE PATHOGENESIS OF OSTEOPOROSIS.

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The pathogenesis of falling bone mineral density (BMD) as a universal feature of advancing age is poorly understood[1]. Frequently culminating in the development of osteoporosis, the process is attributable to more than 500,000 fragility fractures occurring every year in the UK Such injuries are associated with great levels of morbidity, mortality and a £3.5 billion cost to the healthcare economy[2].

With age, humans are known to accumulate somatic mitochondrial DNA (mtDNA) mutations in mitotic and post mitotic tissue, and stem cell precursors[3]. Compelling evidence in recent years, particularly that provided by animal models suggests that these mutations are intrinsic to the ageing process[4-6]. We provide evidence for the first time that mitochondrial dysfunction contributes significantly to the failure of bone homeostasis and falling BMD.

We have utilised a mouse model that accumulates mtDNA mutations at 3-5 times the rate of normal mice, consequently ageing and developing osteoporosis prematurely[7], to clearly demonstrate that osteoblasts are vulnerable to mtDNA mutations. We have developed a new quadruple immunofluorescent assay to show that mitochondrial respiratory chain dysfunction occurs in osteoblasts as a consequence ($p < 0.0001$). We show that this mitochondrial dysfunction is associated with reduced BMD in female and male mice by 7 ($p = 0.003$) and 11 ($p = 0.0003$) months of age respectively. Using osteoblasts derived from mesenchymal stem cells extracted from male and female mice with mitochondrial dysfunction aged 4, 7 and 11 months, we demonstrate a vastly reduced capacity to produce new mineralised bone *in vitro* when compared to wild type cell lines ($p < 0.0001$). Exercise was found to have no beneficial effect on osteoblast and whole bone phenotype in this mouse model. It is likely that mtDNA mutations accumulating over a longer time period in human ageing have significantly detrimental effects on bone biology and diminishing BMD.

48. OSTEOPOROSIS AND AGEING AFFECTS STEM CELL DIFFERENTIATION AND MIGRATION

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There is increasing interest in using anabolic factors such as stem cells to augment fragility fracture repair. One of the factors associated with fracture healing is the retention and migration of stem cells to the site of injury (1-3). The aim of this study was to isolate stem cells from osteopenic rats and investigate and compare the CD marker expression, proliferation, migration, osteogenic and adipogenic differentiation. The hypothesis of this study is that the migration of MSCs from young, adult and ovariectomised (OVX) rats will have different proliferation, differentiation and migratory abilities.

CD marker expression of MSCs from young, adult and osteopenic rats was measured using flow cytometry. Proliferation, osteogenic differentiation and adipogenic differentiation was measured using Alamar Blue, ALP expression and Alizari n Red and quantitative Oil red O respectively. Cells were incubated in Boyden chambers to quantify their migration towards SDF1. Data was analysed using a Student t-test where p values < 0.05 were considered significant.

MSCs from all 3 groups of rats had similar proliferation and expression of CD29(>90%), CD90(>96%), CD34(<5%) and CD45(approx 10%). The proliferation rate was also similar. However, interestingly the migration and differentiation ability was significantly different between the MSCs from the 3 groups of rats. The young MSCs were not only better at differentiating into bone and fat, but they also migrated significantly more towards SDF1. MSCs from OVX rats are similar to MSCs from young rats. However when induced to turn into bone, fat and migrate towards SDF1, young MSCs are significantly more responsive than MSCs from OVX and adult control rats. The poor homing ability and differentiation of the stem cells and their retention may result in a reduction in bone formation leading to delayed union in fractures of osteoporotic patients(4).

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49. PTH 1-34 EFFECTS THE MIGRATION AND DIFFRENTIATION OF YOUNG AND OVARECTOMIZED BONE MARROW DERIVED RAT STEM CELLS

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Intermittent parathyroid hormone (iPTH 1-34) increases bone formation via modelling and remodelling mechanisms and as such is used to treat osteoporosis. The actions of iPTH on mesenchymal stem cell (MSCs) may underpin a further treatment option.

We isolated bone marrow derived MSCs from young (WT) and ovarectomized senile (OVX) rats, investigating the effect of intermittent and continuous PTH administration on migration to SDF-1, proliferation and osteogenic differentiation.

MSCs were harvested from the femora of 6-10week old WT rats and 10-13month old OVX rats. Cells were cultured with 25,50 and 100nmMol of PTH 1-34 added to osteogenic media either continuously or intermittently for 6hours in every 72hour cycle. ALP and Alizarin Red assessed osteogenic differentiation, and Alamar Blue- proliferation. Cells were seeded in a Boyden chamber to quantify SDF-1 migration. A student t-test was used to analyse results, and a p value<0.05 considered significant.

ALP and Alizarin Red were significantly increased for WT and OVX groups at 50nmMol of iPTH. Continuous administration at all concentrations reduced calcium phosphate deposition by day 21 in all groups.

In comparison to cells cultured in osteogenic media, 50nmMol of iPTH led to significantly higher ALP and Alizarin Red measurements up to days 10 and 7 respectively (figure 1). There was no change in proliferation between the groups, and PTH had no effect (figure 2.)

WT MSCs not only had improved osteogenic differentiation, but also showed increased migration to SDF-1 in comparison to OVX groups. iPTH led to further increases in migration of both OVX and WT cells.

iPTH increases the osteogenic differentiation and migration of MSCs from both young and ovarectomised rats, though this effect is not dose dependent. Ultimately, the role of iPTH on MSCs may lead to improved bone formation and cell homing capacity-particularly in the context of osteoporosis.

50. THE RELATIONSHIP BETWEEN MINERAL TO COLLAGEN RATIO, ULTRASTRUCTURE AND MECHANICAL PROPERTIES, DIFFERENCES WITHIN A SINGLE SPECIES.

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Bone has a number of different functions in the skeleton including the physical roles of support, protection and sound wave conduction. The mechanical properties, required for these different functions varies and can be achieved by compositional adaption of the bone material, in addition to changes in shape and architecture. A number of previous studies have demonstrated the relationship between mechanical function and mineral to collagen ratio in bones from different species.

The aim of this study is to test the hypothesis that the mineral to collagen ratio is higher in bone with a mechanically harder matrix within a species. The red deer *(Cervus elaphus)* (n=6) was chosen as a model for studying bone with extreme properties. The mechanical properties of the antler, metacarpal bone and tympanic bulla were defined by indentation using a bench-top indentation platform (Biodent). The mineral to collagen ratio was quantified using Raman spectroscopy. The deposition of mineral was studied at macro-level using pQCT.

The results showed that the hardness (Indentation Distance Increase) was lowest in the metacarpal (8.5µm), followed by the bulla bone (9.4µm) and highest in the antler (14.5µm). Raman spectroscopy showed a mineral:collagen ratio of 1:0.10 (bulla), 1:0.13 (metacarpal) and 1:0.15 (antler) for the different bones. This does not follow the more linear trend previously shown between young's modulus and the mineral:collagen ratio. The location of the mineral appeared to differ between bone types with pQCT revealing locations of concentrated density and banding patterns in antler. Interestingly, Raman spectra showed differences in the amide peaks revealing differences in protein structure.

The results reject the hypothesis but also suggest that the organisation of mineral and collagen has an impact on the hardness modulus. We demonstrate that the red deer provides a good model for studying bone specialisation. This work will provide the basis for further investigation into collagen as a controlling factor in mineral deposition.

51. **DEVELOPMENT OF 3D OSTEOPOROTIC MODEL FOR MICRORNA ASSESSMENT AND MANIPULATION**

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Osteoporosis is an international health and financial burden of ever increasing proportions. Current treatments limit the rate of bone resorption and reduce fracture risk, however they are often associated with significant and debilitating side effects. The most commonly used therapies also do not stimulate osteoblast activity. Much current research focus is aimed at the metabolic and epigenetic pathways involved in osteoporosis. MicroRNAs have been shown to play an important role in bone homeostasis and pathophysiological conditions of the musculoskeletal system. Upregulation of specific microRNAs has been identified in-vivo in osteoporotic patients. It is hypothesized that modulation of specific mircoRNA expression may have a key role in future targeted therapies of musculoskeletal diseases. The assessment and analysis of their potential therapeutic use in Osteoporosis is of great importance, due to the burden of the disease.

We have developed a 3D osteoporotic model from human bone marrow, without the use of scaffold. Magnetic nanoparticles are utilised to form spheroids, which provides a closer representation of the *in-vivo* environment than monolayer culture. This model will provide the basis for analysing future microRNA experiments to assess the potential upregulation of osteoblastogenesis without cessation of osteoclast activity.

The results of initial monolayer and spheroid experiments will be presented. Optimisation of the osteoporotic bone marrow culture conditions, involving response to differentiation medias, analysis of adipose and bone markers and cell migration in spheroid culture will be displayed. Quantitative and qualitative results, including fluorescence microscopy and in cell western, assessing the monolayer and spheroid cultures will be presented. The development of a pseudo osteoporosis model from healthy bone marrow will also be discussed. This model will form a basis of future work on miRNA targeting.

52. ARE THE CRACKS STARTING TO APPEAR IN BISPHOSPHONATE THERAPY?

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Osteoporosis is a global health issue with 200 million people suffering worldwide and it is a common condition in the elderly. Bisphosphonates including alendronate and risendronate are considered as the first line treatment for osteoporosis. However, there is increasing evidence that bisphosphonate (BP) therapy is associated with atypical fractures. Animal studies have reported a dose-dependent association between the duration of BP therapy and the accumulation of micro-damage. We tested the hypothesis that hip fracture patients treated with BP exhibited greater micro-damage density than untreated fracture and 'healthy' aging non-fracture controls.

Trabecular bone cores from patients treated with BP were compared with patients who had not received any treatment for bone metabolic disease (ethics reference: R13004). Non-fractured cadaveric femora from individuals with no history of bone metabolic disease were used as controls. Cores were imaged in high spatial resolution (~1.3μm) using Synchrotron X-ray tomography (Diamond Light Source Ltd.) A novel classification system was devised to characterise features of micro-damage in the Synchrotron images: micro-cracks, diffuse damage and perforations. Synchrotron micro-CT stacks were visualised and analysed using ImageJ, Avizo and VGStudio MAX.

Our findings show that the BP group had the highest micro-damage density across all groups. The BP group (7.7/mm^3) also exhibited greater micro-crack density than the fracture (4.3/mm^3) and non-fracture (4.1/mm^3) controls. Furthermore, the BP group (1.9/mm^3) demonstrated increased diffuse damage when compared to the fracture (0.3/mm^3) and non-fracture (0.8/mm^3) controls. In contrast, the BP group (1.9.mm^3) had fewer perforations than fracture (3.0/mm^3) and non-fracture controls (3.9/mm^3).

BP inhibits bone remodelling, thereby reducing the number of perforated trabeculae, but over-suppression leads to micro-damage accumulation. Accumulated damage could weaken the trabecular bone in the femoral head and neck, increasing the risk of a fracture during a trip or fall.

Late - Afternoon Session: Debates Chamber

INVITED PUBLIC LECTURE

CHAIRING: DR M. BIRCH

KEYNOTE: GRAVITY WAVES, STEM CELLS AND BONE REGENERATION

PROF STUART REID
Professor Experimental Physics, University of the West of Scotland.

POSTERS

53. Multiscale mechanobiological simulation of structural changes as a result of bone remodelling

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Abstract. Macroscale simulation of bone remodelling process is possible to predict how bone skeletal changes that could be affected by bone diseases such as osteoporosis (OP). The results derived from numerical studies could be used as a low cost and efficient technique for better prognoses in clinics. Currently, there are large number of researches focusing on the bone remodelling process at macroscale. Only a limited number of studies introduced the bone cellular structure such as osteocytes, osteoclasts and osteoblast into the computational model. However, bone remodelling is a complex mechainco-biological process which involves structural changes across multiple length scales. It is vital to understand the roles of individual cell during remodelling process and how these cellular factors at microscale affect material heterogeneity at macroscale.
In our study, we aim to investigate different finite element models which have the abilities to simulate bone remodelling process at both macroscale and microscale. The study also determines if the same mechanobiological principles as those proposed at macroscale could be applied directly to investigate the developmental process of bone's microstructure and its material heterogeneity at microscale. The models developed in this study could be used as a predictive tool in understanding the structural changes due to degenerative bone diseases such as osteoporosis.

54. A COMPARISON OF STIFFNESS AND FLEXIBILITY SPINAL TESTING PROTOCOLS

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Back pain is a prevalent condition resulting in an annual cost of £500 million to the NHS. Back pain often arises from the intervertebral disc and can originate from mechanical factors (Adams and Dolan, 2005). A substantial body of research, therefore, is dedicated to the biomechanical investigation of the intervertebral discs (IVDs) (Gardner-Morse and Stokes, 2003). This research field has polarised into two competing protocols: stiffness and flexibility testing. From a mechanical point of view the two are equivalent for elastic specimens. This does not hold true for non-elastic specimens, such as IVDs. The extent of any expected differences is not documented in the literature.

This study investigated the effect of the two competing protocols on the reported properties of IVDs using a custom developed six-axis spine simulator (Holsgrove et al, 2014). A biomechanical phantom of the IVD, comprising nylon blocks joined by a nitrile rubber disc was manufactured. The phantom was subjected to a stiffness protocol characterised by displacements of ±0.5mm for anterior-posterior and lateral shear, ±0.35mm for axial compression and ±1.5deg for all rotational axes. The resulting loads were applied to the specimen subjected to the flexibility protocol. Two stiffness matrices were calculated and the diagonal elements compared. Compared to flexibility testing, stiffness testing resulted in lower values for the anterior-posterior and lateral axes, 66.0±0.9N/mm vs 71.0±7.1N/mm and 55.8±1.5N/mm vs 59.0±2.6N/mm, respectively. The axial axis exhibited an opposite trend: 311.8±17.7N/mm vs 120.2±16.1N/mm. Flexibility testing resulted in lower values for all rotational degrees of freedom. Differences were also recorded for the shape and linearity of the load-displacement curve and for the area enclosed by the curve.

The two testing methods produce data that cannot be easily compared. This important result demonstrates the need to standardise the protocols used to perform biomechanical studies of the spine to ensure comparisons can be made across laboratories.

55. FIBRONECTIN-BASED MICROENVIRONMENTS TO UNDERSTAND THE MIGRATION AND BIOMECHANICS OF CANCER CELLS

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Polymers have become a fundamental tool in cellular biology by mimicking in vivo conditions such as tissue stiffness and extracellular matrix properties found in vivo. The aim of this study was to use polymers to gain further understanding in the cellular biomechanics processes involved in cancer cell adhesion and migration.

In this study fibronectin (FN) coated poly acrylates (poly(ethyl) acrylate (PEA), which has been shown to induce material-driven fibrillogenesis, and poly(methyl) acrylate PMA) are used to understand the behaviour of DU145 human prostate cancer cells. These polymers have a similar structure with the exception of a longer side chain on PEA. This affects their mechanical properties, with PMA being stiffer, and their effect on FN upon its adsorption.

Fibronectin adopts a fibrillar and globular conformation when coated on PEA and PMA, respectively. This has a significant impact on cell response as the RGD cell binding domain and PHSRN synergy sequence of FN are exposed differently depending on the conformation. Here we assess how this affects cell migration, the formation of focal adhesions and drug resistance. Results have shown that cells assemble more focal adhesions on PEA. This however does not translate into differences in migration or resistance to a cytotoxic drug (docetaxel). Other avenues include assessing the phosphorylation of FAK, the resistance to a cytostatic drug (PND-1186), as well as cellular biomechanics.

The study of traction forces of cells on FN coated PEA and PMA have shown that cells exert more forces on PMA compared to PEA. Which is due to the stiffer surface of PMA, but could potentially be linked to the difference in conformation of FN on each surface and how cells interact with it.

These results suggest that using polymers to control differences in FN conformation can affect how these cells interact with their environment.

56. FUNCTIONALIZATION OF PEEK TO MAXIMIZE THE EFFECT OF BMPS

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Mechanical properties of PEEK are the most interesting characteristic about this material. However, PEEK has to be modified to promote cellular adhesion and differentiation1. Here we present a novel bioactive PEEK coating that involves a layer of HA on which a poly ethyl acrylate polymer (PEA) that assembles fibronectin (FN) and promotes high efficient presentation of growth factors for cell adhesion, growth and differentiation 2.

First PEA polymer was solubilized in 8% toluene; this was used to spray onto PEEK samples. Then, FN was adsorbed on the sample from a solution of 20 µg/ml. 25 ng/ml BMP2 & BMP7 coatings were performed right before cell cultures. Samples were characterized to determine the surface roughness (with and without PEA) using AFM and SEM. Water contact angle was measured before and after FN coatings. An hMSCs culture was performed to proof cell adhesion and study the morphology on the different surfaces. For differentiation studies, 5 and 21 days hMSCs cell culture were performed and gene expression was analyzed.

Roughness on the PEEK/HA PEA treated decreased compared with the PEEK/HA samples. The surface of the original sample was covered partially, leaving some HA available. WCA was studied before and after FN coating on all the samples. PEEK/HA samples coated with PEA and FN had a 60º, while WCA on PEEK/HA after FN coating was 15º. Cell morphology on PEEK/HA samples was more rounded, while on PEA treated samples the cells were more flattened and spread.

Coating PEEK/HA samples partially with PEA and FN have proven to be an innovative and easy surface treatment. The original hydrophobicity and roughness of the PEEK/HA surface was decreased, where cell adhesion and differentiation properties offered by FN fibrillogenesis on PEA are taken in advantage. Here we show an easy translational technique to improve biological PEEK properties for being used in dental implants.

57. PRESENTATION OF INTEGRIN/GROWTH FACTOR BINDING DOMAINS BY PLASMA POLYMERISATION AND ITS EFFECT ON MESENCHYMAL STEM CELL ADHESION AND DIFFERENTIATION

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The surface modification of orthopaedic implant materials is important in facilitating osseointegration at the implant/tissue interface. Chemical modification is one of the many ways of imparting bioactivity onto a material surface and is known to have a potent effect on cell behaviour. Various types of biomolecules, such as proteins, peptides, and growth factors (GFs), have been used to improve cell-material interaction via surface modification. For example, fibronectin (FN) is an extracellular matrix protein known to regulate cell adhesion. The structural domains of FN allow it to bind to other FN molecules as well as a variety of GFs, including bone morphogenetic protein-2 (BMP-2). BMP-2 plays important roles in bone and cartilage development, among which is the differentiation of mesenchymal stem cells (MSCs) into the osteoblastic lineage.
In our work, we use synthetic polymers with the ability to induce conformation changes in FN structure. Poly(ethyl acrylate) (PEA) is known expose the integrin- and GF-binding domains of FN by promoting its organisation into nanonetworks from its native globular form, thereby allowing efficient and synergistic signalling between integrins and GFs. Here we employ an inductively coupled plasma polymerisation system to present thin coatings of PEA on 2D surfaces and study the adsorption of FN on PEA-modified surfaces. The synergistic interaction of BMP-2 with the exposed domains of FN on plasma-polymerised surfaces is also evaluated, followed by studies of MSC adhesion and differentiation at the protein-GF interface. Our system provides a simple and effective method of presenting FN on a material surface by increasing the availability of its binding domains. The plasma system can also be applied to 3D synthetic materials for orthopaedic purposes.

58. IMPACT OF TISSUE ARCHITECTURE ON THE DEVELOPMENT AND PHYSIOLOGY OF SKELETAL MYOBLASTS

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Matrix rigidity emerged recently as a major regulating cue of both tissue development and of a plethora of pathophysiological conditions, including degenerative diseases and cancer. Cells are able to respond to physical as well as biological signals of their environment mainly by reorganising their cytoskeleton leading to alterations of their morphology and adhesion to extracellular matrix. Specifically for skeletal myoblasts, the elasticity of the cellular microenvironment influences not only cell adhesion and contraction, but also their differentiation into myotubes. This study aims to explore the use of a polyacrylamide hydrogel system with tunable mechanical properties and protein availability to investigate the behaviour of skeletal myoblasts on substrates of varying biophysical properties. C2C12 cells were cultured on polyacrylamide hydrogels of soft (0.7 kPa), medium (7 kPa) and rigid (38 kPa) stiffness coated with fibronectin. We assessed adhesion of cells on the hydrogels as well as expression of differentiation markers. We hope to highlight the impact of matrix stiffness on cell phenotype and behaviour. Identifying the optimal balance between cell adhesion remodelling and substrate compliance will improve skeletal muscle tissue engineering and could potentially refine current clinical practice for the treatment of several muscle degenerative diseases.

59. GRADED ORGANISATION OF FIBRONECTIN ON POLYMER SURFACES TO TUNE MESENCHIMAL STEM CELLS RESPONSE

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Cells receive biochemical and mechanical signals from the surrounding environment, the extracellular matrix (ECM). An essential component of the ECM is fibronectin (FN) which contains multiple domains for binding to other ECM proteins, other FN molecules, growth factors and cell receptors. FN is synthesised by most cells and it is assembled into a network via interactions with cell receptors. In order to gain insights into the cell-ECM interplay, a large body of research aims at engineering biomaterials that recapitulate the properties of the ECM. It is known that the chemical properties of surfaces influence FN conformation as well as cell response. To this end, a large number of growth factors involved in bone tissue engineering have been used. It has been previously shown that poly(ethyl acrylate), PEA, induces FN fibrillogenesis upon adsorption, a process that triggers a network-like organisation of FN. In contrast, globular aggregates were observed on poly(methyl acrylate), PMA, which differs from PEA in a single methyl group.

In this work, we explore the potential of surfaces with similar chemistries (PEA and PMA) in investigating FN fibrillogenesis. Using a series of copolymers with controlled ratio of EA and MA, we investigated the conformation and the amount of FN upon adsorption. Additionally, we characterised the availability of important binding sites of FN using monoclonal antibodies as well as the amount of BMP2 adsorbed on the FN coated surfaces. In order to study how FN conformational changes regulate cell behaviour, we characterised the adhesion of human mesenchymal stem cells (hMSCs). In order to assess the osteogenic differentiation of hMSCs, we studied the expression of the marker osteocalcin after using BMP2.

This work aims at elucidating how FN fibrillogenesis can be controlled in a surface-dependent way and how such changes can determine cell behaviour and, particularly, the osteogenic differentiation of hMSCs.

60. USING SONOTWEEZERS FOR CONTROLLED CELL 3-D PATTERNING IN COLLAGEN HYDROGELS.

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A core approach in current tissue engineering involves the fabrication of constructs designed to mimic key elements of the natural surrounding cell environment with incorporated alterations capable of enhancing regenerative potential. Collagen; a dynamic, self-assembling, fibrous material is found in abundance in the extracellular matrix. Its roles in directing cell fate through chemical interactions, organisation, orientation and fibre structure are vast and well documented. Synthesis of collagen hydrogel matrices can be achieved readily in the laboratory and fashioned to specific standards simply through pH and temperature adjustments. Further modification possibilities such as cell seeding and growth factor inclusion make the effectiveness and popularity of collagen hydrogels as biomaterials for the creation of tissue scaffolds understandable.

Surface acoustic waves have previously been employed as a non-destructive means to sort and pattern cells two-dimensionally on substrates with moderate complexity. Using the octagonal sonotweezer device created here at University of Glasgow we have developed a one-stage procedure for the three-dimensional in-situ alignment of hTERT cells in a collagen hydrogel during the gelling process. Cells are held soundly in the matrix and can be seen spreading to adhere together in long strands directed in the pattern during setting and remained through the extent of the 7 day culture period.

Our aim is to use this procedure to gain an insight into the nature of the stresses imposed on the ECM by adhering and migrating cells through monitoring the impact of cultures in overall hydrogel shape. Further work will involve transferring this procedure to a more neuronal orientated approach using 3-D aligned Schwann cells in collagen for rapid scaffold creation promoting the guidance of regenerating axons in the peripheral nervous system.

61. A METHOD FOR ASSESSING CHONDROCYTE VIABILITY USING CONFOCAL LASER-SCANNING MICROSCOPY IN MURINE MODELS

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Maintenance of healthy articular cartilage relies upon sophisticated interactions between chondrocytes and the extracellular matrix. Fluorescence-mode confocal laser scanning microscopy (CLSM) is a diverse experimental technique that permits the assessment of undisturbed in situ cells without the need for physical sectioning. CLSM has been used widely in orthopaedic research with the majority of studies being conducted on cartilage explants. Murine models are widely employed in arthritis research and have advantages over explant models because of the ability to manipulate disease pathophysiology and study treatments in-vivo. We have developed a novel technique for studying chondrocyte viability using CLSM examination of murine stifle joints.

Mouse stifle joints are carefully dissected immediately after sacrifice. Specimens are incubated for 1hr at 21˚C in penicillin (100 U/ml) and streptomycin (100mg/mL) containing Dulbecco's modified eagle medium with 30µM 5-chloromethylfluorescein diacetate (CMFDA) and 15µM propidium iodide (PI). Explants are subsequently fixed overnight in 4% formaldehyde prior to storage at 4˚C in 1% PBS. The explants are then secured onto a petri dish with blu-tack and submerged in 1% PBS ready for analysis. An upright Zeiss LSM510 Axioskop (Carl Zeiss Ltd.) CLSM, fitted with an x10/0.3 dry objective is used to acquire optical sections of CMFDA and PI labelled in situ chondrocytes within the patellofemoral groove of the femur. The LSM file generated from the confocal microscope is then analysed using IMARIS x64 version 8.0.2 (Bitplane AG).

Using this technique we have been able to consistently image healthy articular cartilage without chondrocytes damage during the preparation of samples. We have been able to induce both focal areas of cell death (with scalpel or needle injuries) and global cell death (through sample drying or a septic arthritis model). This technique may have applications for other research groups studying cartilage physiology, pathology and repair.

62. A RETROSPECTIVE ANALYSIS OF IMPLANTS PLACED IN BONE GRAFTED SITES.

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Implant treatment for the replacement of missing teeth is considered the gold standard. However, implant placement requires sufficient bone in the surgical site. Autogenous bone grafting overcomes this problem but can result in donor site morbidity. Implants have a very high success rate but are reported as less successful when placed within grafted sites. The aim of this study was to investigate the success of implants placed in grafted sites over a period of five years within a single surgical unit.

A retrospective analysis of forty patients who had implants placed four-five months after bone grafting (n=36) or on the same day of grafting (n=6). Restorations were placed 6-7 months following implant placement. 149 implants were placed in 15 males and 25 females, aged 18 to 65 (mean 28).

The reasons for implant placement were; hypodontia (n=24), loss of teeth due to trauma/pathology (16). Two patients were smokers. The grafts used were iliac crest (n=22) or mandibular symphysis (n=18) combined with Bio-Oss® and BioGuide®. Branemark implants were used in 30 patients and Straumann implants in 10. 127 implants were placed in the maxilla and 22 in the mandible. A mandibular symphysis graft failed in one patient. Three patients who had mandibular symphysis grafts complained of temporary paraesthesia in the lips. Two patients with iliac crest grafts complained of paraesthesia in the donor site. They had slight walking difficulties, which improved after two months. 5 implants failed (97% success rate) within six months of placement; maxilla (n=5). Of these, two were placed in mandibular grafts, two in a combination of mandibular and iliac crest grafts, and 1 in an iliac crest graft.

This study highlights the need for bone substitutes to eliminate the complications associated with harvesting autogenous bone grafts.

63. MEDIAL SOFT-TISSUE RELEASE FOR A LATERALIZING CALCANEAL OSTEOTOMY

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A lateralizing calcaneal osteotomy (LCO) for pes cavus is generally regarded to be harder to shift than a medializing calcaneal osteotomy for pes planus. LCO can also cause a significant reduction of tarsal tunnel volume and some surgeons recommend releasing the tarsal tunnel routinely.

Determine all the structures which restrain a lateral shift in lateralising calcaneal osteotomies using a cadaveric study.

Permissions were obtained to dissect 8 embalmed below-knee cadavers. LCO was performed on 4 cadavers using a standard lateral approach, and the lateral shift was measured before and after the release of tarsal tunnel. However, our approach changed due to our findings after the first 4 cadavers.

We found no significant change in lateral shift before and after tarsal tunnel release. We performed further dissection around the osteotomy and found the Abductor hallucis muscle to be the main restraint to a lateral shift. We changed the method in the subsequent 4 cadavers to LCO with abductor hallucis fascia and plantar fascia release, instead of tarsal tunnel release. By releasing the fascia over Abductor hallucis muscle as well as the plantar fascia, it was possible to increase the lateral shift in LCO by at least another 5mm on average.

Limitation of lateral shift with LCO is generally considered to be due to tight soft-tissues in pes cavus, and several variations of LCO are practiced to overcome this limitation. However, no attempt has been made so far to identify any particular structure contributing to the limitation of lateral shift.

Our study suggests that the Abductor hallucis muscle the main structure limiting lateral shift in LCO, and release of the fascia over the abductor hallucis as well as the plantar fascia should be an essential part of the lateralizing calcaneal osteotomy.

64. IN VIVO EVALUATION OF PORCINE PATELLA TENDON AS AN APPROPRIATE EXPERIMENTAL MODEL FOR TENDON SCAFFOLD IMPLANTATION

Bryan TH Koh.

The use of tissue-engineered or synthetic tendon scaffolds to augment the reconstruction of tendons has been widely studied in a variety of animal models. Despite similarities in the methodology used, it is difficult to compare outcomes due to the differences in the animal models used. Hence, the purpose of this study was to evaluate the suitability of the micro-pig for tendon reconstruction research.

Six fully matured male micropigs over 1 year old, between 20-25kg were selected for this study. The knee joint was approached anteriorly and dissected in layers until the patella tendon was exposed. The length, Anterior-Posterior (AP) diameter and Medial- Lateral (ML) diameter of the patella tendon was measured. A 15mm surgically created defect was created at the midpoint of the patella tendon, which was reconstructed with 2 tubular shaped synthetic scaffolds. The micropigs were sacrificed at 4 weeks and 12 weeks post-op.

The gross morphology of the micropig patella tendon was similar to that of humans. It is a thick, flat tendon measuring on average 49.5mm in length, 5.0mm Anterior-Posteriorly (AP) and 12mm Medial-Laterally (ML) at 30° of flexion. Necropsy revealed no complications such as suture breakage or suture pullout through the patella tendon.

The reproducibility of the micro-pig makes it an attractive model to establish consistency when comparing the effectiveness of surgical techniques and tendon implants. The patella tendon of the micropig is a reliable tendon to use for tendon reconstruction research.

65. DEVELOPMENT OF AN ORTHOPAEDIC FUNCTIONAL OUTCOME MEASURE PACKAGE

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Four important outcomes following total knee arthroplasty (TKA) are knee range of motion (ROM) and strength, gait kinematics and walking stability. Current outcome measures used to test these variables however, have been reported as unreliable and inaccurate. According to recent research, three-dimensional motion analysis is the most effective tool for detecting changes in the function of the knee joint pre- and post-operatively. Nevertheless, *clinical* use of this technology is currently difficult.

The first aim of this project was therefore to design a small-scale and clinic-appropriate motion capture system. The second aim was to create a simple software package which could be used with the system to assess functional outcomes of TKA patients.

Our bespoke motion capture set-up consists of a treadmill which is immediately surrounded by two frames, onto which motion capture cameras are mounted. This set-up has a significantly smaller footprint than the average gait laboratory, and is also moveable; a function which is paramount for a hospital environment, where rooms are often multi-functional. The software which has been developed to complement this set-up enables the user to check whether the cameras are correctly calibrated before patient arrival, potentially saving preparation time. On arrival, patients are required to wear clusters of markers over their clothing. Following calibration, ROM and strength assessments can be carried out on the treadmill. These assessments have been designed to be similar to the clinical standard methods (goniometer- and myometer-based assessments) to maintain acceptability. Gait kinematics can then be recorded with the bespoke software and treadmill (No data processing required by clinician). A further application can then be used to investigate cycle-to-cycle stability following walking trials. A recent validation study showed similarities between the standard tools and bespoke software ($p > 0.05$), suggesting that the results obtained with these alternative methods are clinically acceptable.

66. NOVEL TUNED ULTRASONIC NEEDLE FOR BONE BIOPSY

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Bone biopsy is an invasive clinical procedure. A core-needle, or trephine, biopsy is utilised in order to preserve bone architecture, facilitating diagnostic immunohistochemistry. Trephine bone biopsy is reported to be painful and often causes significant soft tissue damage. We developed a novel ultrasonic biopsy needle to penetrate hard tissue and minimise damage.

The ultrasonic device utilizes a full-wavelength resonating needle, optimised for maximum amplitude with minimum bending using finite element analysis (FEA). The results of the FEA were confirmed by experimental modal analysis using a 3D laser Doppler vibrometer.

Biopsies were taken from the metaphysis and diaphysis of ovine femoral bone using both the ultrasonic device and with existing manual trephines. The ultrasonic needle was used with a vibrational amplitude of 80µm. No additional force was applied to the device during biopsy, however a small coolant flow was used to prevent overheating. Micro-architectural analysis of samples from both devices was performed by µCT.

Forces in excess of 500N and a significant twisting motion, resulting in a conical orbit of the needle, were required for diaphyseal biopsy with the manual device. This resulted in damage to the sample and increased the hole diameter. It was considered that this motion could result in soft tissue damage, often seen as a large haematoma post biopsy.

Examination of the biopsies showed that the ultrasound device extracted higher quality samples from the thick diaphyseal bone, which the manual device struggled to penetrate. The manual device caused less breakage in the trabeculae extracted from the metaphysis. However both devices could perform a satisfactory metaphyseal biopsy.

This work shows the potential of this novel device to extract samples of bone, minimising damage to the sample, and due to no operator force and tool movement, minimise soft tissue damage in the patient.

67. BIOCHEMICAL MARKERS OF EARLY MORTALITY IN PATIENTS WITH PATHOLOGICAL HIP FRACTURES DUE TO METASTATIC DISEASE

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There is comprehensive data in the literature addressing the 6 to 18-month survival in patients with pathological neck of femur (NOF) fractures due to bony metastases. However, little is known about the early mortality in this group.

The aim was to quantify the 30 and 90-day mortality in patients with pathological NOF lesions/fractures and identify any biochemical markers associated with early death.

Orthopaedic trauma lists over one year were used to identify all patients with a pathological NOF fracture/lesion. Survival, surgical and biochemistry data was compared to controls with non-pathological fractures.33 patients had a metastatic NOF lesion/fracture. They were compared to an age and gender-matched control group of 33 patients with non-pathological NOF fractures.

Delay from referral to surgery was comparable between NOF fractures in the pathological and non-pathological groups. Within the metastatic group, delay was higher in patients with a fracture compared to a pathological lesion (average 7.4 and 0.6 days respectively, $p<0.05$)).

Mortality was higher at 30 and 90 days in the pathological lesion/fracture group compared to controls (15% 5/33 vs 9% 3/33 $p=0.02$, and 42% 14/33 vs 12% 4/33 $p<0.01$, respectively). Patients who died within 90 days had lower average sodium (135 vs 138, $p<0.05$), creatinine (48 vs 62, $p<0.05$) and APTT (27 vs 32, $p<0.05$). They had a higher average white cell count (11.3 vs 7, $p<0.05$) and c-reactive protein (55 vs 18, $p<0.01$). Patients with metastatic lesions and early mortality also had lower albumin (20 vs 30, $p<0.01$) and haemoglobin (102 vs 121, $p<0.01$), which were significantly higher in the control NOF group who died early (28 and 118 respectively, $p<0.05$).

Patients with pathological NOF lesions have multiple biochemical abnormalities associated with early mortality. We propose a prospective study aimed at measuring whether correction of these abnormalities can improve early survival in this group.

68. COMPUTER ASSISTED PLANNING IN CORRECTIONS OF FOREARM SHAFT DEFORMITIES

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Standard treatment for mal-union of forearm shaft fractures with corrective osteotomy is a challenging process, due to the complex 3D deformity seen in some cases. Computer assisted pre-op surgical planning with production of patient specific surgical guides and implants are becoming logistically practical and are proposed to increase the precision of corrections. Use of computer assisted planning for distal radius fractures is well documented however use of this technique in forearm shaft fracture mal-union is less common. Very encouraging outcomes from other centres have been reported recently and we report on our experience trialling this technique in a single case.

The patient had high velocity injury resulting in both forearm bone fracture with ongoing left radius non-union and three previous attempts at internal fixation and grafting. He presented with a broken plate after minor injury and was diagnosed with non-union, decreased radial bow, mal-rotation and shortening of the radius with a 1 inch bone defect. He remained functionally restricted with minimal pronation.

Pre-operative Multi-detector CT scan of the normal and affected forearm were taken and a virtual 3D reconstruction was made. Software from Materialise NV (Leuven, Belgium) was used for virtual planning. A 3D anatomical model of the deformity was produced. The 3D correction was planned with autologous bone graft for the defect. A standard LC DCP plate was used for fixation.

We found adopting this novel technique to be more predictable and more precise than our standard surgeon led osteotomy technique in complex cases. After the detailed pre-op design stage analysing the entire deformity, the exact planned corrective surgery was smoothly executed, effortless, and achieved almost anatomical accuracy compared to previous osteotomies. We await his follow up to see how his function improves.

69. THE EFFECT OF HIP JOINT ANGLE ON QUADRICEPS MUSCLE TORQUE IN HEALTHY SUBJECTS.

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Accurate muscle-performance evaluation is crucial for establishing the effectiveness of orthopaedic interventions and rehabilitation programmes. Current practice tends to assess and train the quadriceps muscles in a sitting position, but this may be inadequate and misleading due to differences between the evaluation in the sitting position and the functional position and requirements of certain sports. This study aimed to determine the effect of changes in the hip-joint position on quadriceps muscle torque (concentric and eccentric) and to explore the gender effect. To determine whether a relationship exists between quadriceps torque and rectus-femoris length at different hip positions.

A crossover design investigated the effect of hip-joint angle on quadriceps torque. Concentric and eccentric quadriceps muscle contractions were measured in three different hip positions, Supine lying (hip10 degrees flexion) half sitting (55 degrees flexion, and upright sitting (85 degrees flexion) and a correlational analysis explored the relationship between quadriceps torque and rectus-femoris length. Participants:Twenty-eight healthy volunteers were recruited following a sample-size calculation (G* Power V3.1) (Age = 28.39 ±4.63 years, Body Mass Index = 26.11 ± 4.29). Ethical approval was obtained from the Cardiff University School of Healthcare Studies Research Ethics Committee. Biodex 3 measured quadriceps torque and data was normalized to bodyweight.

There were significant differences in the quadriceps muscle's concentric and eccentric torque between lying supine and regular sitting position, but not between any other hip positions, with the sitting position for both concentric and eccentric recording the greatest levels of torque (1.78 and .99 Nm/kg),with no significant difference between males and females, and no significant correlation between quadriceps torque and rectus-femoris length.

The hip-joint angle significantly affects quadriceps concentric and eccentric contraction. A sitting position may be used for training to maximize tension generation.

70. A NOVEL METHOD FOR BIOCOMPATIBILITY TESTING OF ORTHOPAEDIC WEAR DEBRIS USING THREE DIMENSIONAL PARTICLE EMBEDDED AGAROSE GELS

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Currently, different techniques to evaluate biocompatibility of orthopaedic materials, including 2D cell culture for metal and ceramic wear debris and floating 2D surfaces or 3D agarose gels for UHMWPE wear debris, are used. We have developed a single method using 3D agarose gels that is suitable to test the biocompatibility of all three types of wear debris simultaneously.

Clinically relevant sterile UHMWPE and CoCr wear particles were generated using methodologies described previously [1,2]. Commercially available nanoscale and micron-sized silicon nitride (Si_3N_4) particles (<50nm and <1μm, Sigma UK) were sterilised by heat treatment for 4h at 180°C. Agarose-particle suspensions were prepared by mixing warm 2% (w/v) low-melting-point agarose solution with the particles dispersed by sonication in DMEM culture media. The suspensions were then allowed to set at room temperature for 10min in 96 well culture plates. Sub-confluent L929 murine fibroblasts were cultured on the prepared gels for up to 6 days in 5% (v/v) CO_2 at 37°C. After incubation, the viability of cells was measured using the ATP-lite assay; the results were expressed as mean ± 95% confidence limits and the data was analysed using one-way ANOVA and Tukey-Kramer post-hoc analysis.

The gels were observed to ensure uniform distribution of particles and migration of cells into the gel. No significant reduction in viability was observed for nanoscale and micron-sized Si_3N_4 particles at low doses (0.5μm^3 per cell) and high doses (50μm^3 per cell), or for UHMWPE wear debris at high doses (100μm^3 per cell).

Moreover, the viability was significantly reduced for high doses of CoCr wear debris (50μm^3 per cell) and the positive control, camptothecin (2$\mu g.ml^{-1}$) at day 6 [Figure1]. These results are consistent with the literature [2,3] and therefore validate our 3D agarose cell culture method for comparing cytotoxicity of polymer, metal and ceramic particles in a single assay, simultaneously.

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71. MICROSTRUCTURAL ANALYSIS OF DISUSE-RELATED OSTEOPOROSIS IN AN ANIMAL MODEL OF SPINAL CORD INJURY

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Disuse-related bone loss is a consequence of spinal cord injury (SCI). Extensive paralysis and immobilisation after SCI leads to an increased risk of fracture in the long bones (tibia, femur). A better understanding of disuse-osteoporosis and improved diagnostics are necessary before effective rehabilitation strategies can be designed; to attenuate or reverse bone loss post-SCI. Due to the complexities of SCI in human patients, greater understanding of the underlying mechanisms and effects of disuse-osteoporosis can be determined using animal models.

Description of the micro-architectural changes that occur in the limbs of a rodent model of complete SCI.

Sixteen young male Wistar rats (Harlan, UK) (body mass 200-250g) were assigned randomly in to two groups. One group was given a transection of the spinal cord at thoracic level T9 (n=8), while the other group were sham-operated (n=8). 16-weeks post injury all rodents were sacrificed. Subsequently micro-Computed Tomography (microCT) scans of the distal femurs were taken at 70 KVp and 6.89 µm voxel size. Scaled volumes of interest in the distal metaphyseal trabecular and mid-diaphyseal bone were selected. Trabecular and cortical bone morphometric parameters were quantified and compared. The trabecular morphometric parameters analysed were trabecular bone volume fraction; mean trabecular thickness, separation and number; structural model index; trabecular pattern factor; degree of anisotropy and trabecular extent. The cortical morphometric parameters analysed were total cross-sectional area inside the periosteal envelope, cortical bone and medullary canal areas and mean cortical thickness.

Compared to the control group, SCI appeared to lead to: i) 42% lower trabecular volume ($p<0.001$), characterised by 44% reduction in trabecular number ($p<0.001$), ii) 2% longer bones ($p = 0.037$) and iii) 15% smaller bone cross-sectional areas ($p<0.001$).
This study indicates that T9 transection leads to longer, thinner and potentially weaker distal femurs, and provides a useful model for disuse osteoporosis in SCI.

72. PRE-TREATMENT OF HUMAN DENTAL PULP STEM CELLS WITH NOVEL HISTONE DEACETYLASE INHIBITORS TO ENHANCE BONE TISSUE ENGINEERING EFFICACY

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The increasingly ageing population suffers from bone damage caused by trauma, cancer, or age-associated diseases. The ability to generate new bone is still a major clinical need. The key for bone tissue engineering strategies is to effectively control/divert mesenchymal stem cell (MSC) differentiation down the osteogenic lineage. Histone deacetylases (HDAC) play a key role in epigenetics. A number of studies showed that MSCs gene expression can be manipulated by controlling the coiling and uncoiling of DNA around histones without altering the genome and the inhibition of HDAC3 has been linked to osteogenic differentiation. Therefore, this study aims to evaluate the effects of a novel HDAC3 selective inhibitor MI192 on the cell viability and osteogenic differentiation of human dental pulp stem cells (HDPSC).
The cell viability was evaluated using MTS assay after cells were treated with MI192 at 1, 5, 10, 20, 50µM over 24, 48 and 72 hours. Treatments with MI192 at ≥ 20 µM after 24 hours, ≥ 5 µM after 48 hours, or ≥ 1 µM after 72 hours significantly reduced the cell viability. These results were confirmed using PicoGreen DNA quantification assay. In further studies, HDPSCs cultured in monolayer were treated with MI192 at 1, 2, 5 µM for 48 hours prior to osteogenic culture for 14 days and alkaline phosphatase (ALP) was examined by histological staining and biochemical quantitative assays. The ALP expression was significantly enhanced in cells pre-treated for 48 hours with 2 µM MI192 compared to non-treated cells and cells treated with 1 µM MI192. Similarly, enhanced expression of ALP was observed in 3D pellet culture from MI192-treated HDPSCs compared to non-treated controls.
Our results demonstrate the potential of using MI192 to enhance the efficacy of bone tissue engineering.
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73. COMPARISON OF NEEDLE PROBE AND COMPUTED TOMOGRAPHY IMAGING TECHNIQUES FOR THE MEASUREMENT OF ARTICULAR CARTILAGE THICKNESS IN THE ANKLE

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Arthritis of the foot and ankle affects approximately 4% of the global population. Identifying the characteristics of natural cartilage is important for understanding the degeneration pathway. Several methods have been reported for evaluating cartilage thickness, from shadowgraph visual analysis through to CT-scanning, with limitations highlighted for all methods. The aim of this study was to compare a destructive needle-probe test against a non-destructive CT-imaging technique, for in-vitro measurement of cartilage thickness.

Osteochondral pins were extracted from the talus and tibia surfaces of porcine legs (n=16; 4 pins per leg). Pins were placed in a bespoke holder which allowed thickness measurements to be recorded in the same position on each pin for both methods. CT images of the pins were acquired and thickness measurements recorded for three regions on each pin. The needle-probe method recorded the load response as the needle was inserted into, and through, the cartilage and thickness determined by analyzing load response changes associated with the cartilage surface and cartilage-subchondral bone interface.

The mean cartilage thickness was significantly higher when measured by needle-probe methods compared with the CT method. There was a positive correlation between both thickness measurements. Across all samples and surfaces, the mean thickness measured by needle-probe method was 0.81±0.13mm and 0.60±0.13mm for the CT method.

The needle-probe method may tend to over-estimate thickness. Some deformation may occur as the needle penetrates the cartilage and analysis of cartilage thickness depends on identifying the change in load response when the needle encounters the sub-chondral bone, which may be subjective. CT imaging of the pins following needle-probe measurement indicated the needle pierced the sub-chondral bone, thus causing an over-measurement of thickness. Thickness measurements through CT imaging will depend on scan resolution and the ability to differentiate between the calcified zone of cartilage and sub-chondral bone.

74. CONSIDERATIONS FOR THE TECHNICAL DEVELOPMENT OF 405NM LIGHT FOR DECONTAMINATION APPLICATIONS IN ARTHROPLASTY SURGERY

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Post-operative infection rates following arthroplasty surgery are reported to be as high as 4%, with this rate increasing if patients undergo revision procedures. These infections cause serious patient discomfort and trauma, and have major financial implications due to the increased treatment costs, therefore improved methods of infection control are being sought. Recent studies have demonstrated the application of antimicrobial 405 nm violet-blue light for continuous environmental decontamination of hospital isolation rooms, and this work discusses the technical elements that would be required for optimisation of this technology for application during arthroplasty surgical procedures.

Laboratory studies were firstly conducted to establish the differential sensitivity of mammalian osteoblast and bacterial cells, with exposure times selected to reflect the duration of routine arthroplasty procedures. Cell viability results demonstrated a critical dose of 36 J/cm^2 (5 mW/cm^2 for 2 hours) which was non-detrimental to exposed osteoblasts, but exerted a significant antimicrobial effect – up to 100% reduction – of bacterial pathogens, including *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Analysis of existing operating theatre lighting was conducted in order to determine its standard optical output, and to investigate how this could potentially be altered to provide antimicrobial lighting. Two standard white light sources were tested: a halogen light system, and a light-emitting diode (LED) system. The spectral output of both sources highlighted the inclusion of 405 nm light in the emission spectra, although at much lower levels than would be required for antimicrobial activity. This study therefore discusses the potential for technical adaptation of existing operating lighting systems, and development of new lighting systems, which would facilitate 405 nm light decontamination during surgical procedures, thus providing a technology which could potentially be applied as a complementary infection control strategy during arthroplasty surgery.

75. A NOVEL MICROFLUIDIC-BASED PLATFORM TO PRODUCE PROTEASE- DEGRADABLE MICROGELS FOR PROTEIN DELIVERY

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The use of carriers for controlled delivery of specific biological molecules is a promising strategy in regenerative medicine, by increasing the retention of therapeutic molecules at the treatment site to allow cells to migrate to the area of injury and to proliferate and differentiate. Hydrogels, highly hydrated cross-linked polymer networks, have been studied as potential protein carriers due to their high water content, soft consistency, high porosity and biocompatibility. In addition, they can be easily injected into the body as microgel suspensions. Nevertheless, systems that provide tuneable degradation profiles for controlled delivery are still lacking. Poly(ethylene glycol) (PEG) hydrogels present minimal inflammatory reactions, and different functionalities can be easily incorporated to its structure. In this work, we have established a new platform to produce protease-degradable microgel making use of microfluidic techniques to develop a system where the hydrogel degradation rate and subsequently the protein release can be easily controlled.

Microfluidic devices with flow focusing geometry and 200 µm nozzles were used to synthesize monodisperse biocompatible hydrogel microparticles. Two different solutions of macromer 5% PEG-4MAL (20 kDa) and crosslinker (GPQ (W) or VPM peptides) were prepared in PBS at a 2:1 molar ratio. The kinetics of the gelation reaction was controlled with the pH of the solution. Protein encapsulation was carried out by dissolving the desired protein in PEG-4MAL solution before mixing with the corresponding crosslinker solution in the microfluidic device. The release rate of the encapsulated proteins, evaluated by enzymatic degradation via collagenase I and fluorescence, was controlled by the size of the particles, hydrogel network density and the protease-dependent cleavage of peptide crosslinkers, providing a tuneable set of parameters for controlling release kinetics.

The novel platform for controlled protein delivery established in this work provide a versatile tool that can be extended to others applications for regenerative medicine.

76. PROTEASE-DEGRADABLE HYDROGEL FOR SPINAL CORD INJURY REPAIR

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Synthetic hydrogels are widely used for regenerative medicine applications. Polyethylene glycol hydrogel functionalized with maleimide terminal groups (PEG-MAL) provides the advantage of being efficiently cross-linked with protease-degradable peptides at reaction time scales appropriate for its clinical use with in situ gelation, and the ability to incorporate biological molecules such as cell adhesive sequences and growth factors to enhance cell adhesion and differentiation. Additionally, closely matched mechanical stiffness between the gel and spinal cord tissue make this hydrogel a candidate material for injection into the spinal cord. In this study, PEG-MAL hydrogels are characterised, and their capacity to enhance cell adhesion and differentiation along a neuronal lineage is evaluated.

PEG-MAL hydrogels with RGD adhesive peptide were cross-linked with VPM, a protease-degradable peptide. Flat gel samples were prepared in a sandwich-like setup and their mechanical properties were measured with atomic force microscopy. PC12 cells were seeded onto the flat gels surface and cell adhesion was evaluated after 1 day of incubation. Focal adhesions and cytoskeleton conformation were analysed via immunostaining for vinculin and actin staining respectively. PC12 cells derived from rat pheochromocytoma tissue can prompted along a neuronal lineage upon exposure to nerve growth factor (NGF). Hydrogels were synthesised with NGF to investigate the effect on PC12 cell differentiation by staining for the neuronal marker β3–tubulin. Most promising material candidates from this work will be tested in vivo by injection into a contusion injury model of rat spinal cord.

77. SCANNING ELECTRON MICROSCOPICAL OBSERVATION OF AN OSTEOBLAST/OSTEOCLAST CO-CULTURE ON MICROPATTERNED ORTHOPAEDIC CERAMICS.

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In biomaterial engineering, the surface of an implant can influence cell differentiation, adhesion and affinity towards the implant. On contact with an implant, bone marrow-derived mesenchymal stromal cells demonstrate differentiation towards bone forming osteoblasts, which can improve osteointegration. The process of micropatterning has been shown to improve osteointegration in polymers, but there are few reports surrounding ceramics.

The purpose of this study was to establish a co-culture of bone marrow-derived mesenchymal stromal cells with osteoclast progenitor cells and to observe the response to micropatterned zirconia toughened alumina ceramics with 30 μm diameter pits. The aim was to establish whether the pits were specifically bioactive towards osteogenesis or were generally bioactive and would also stimulate osteoclastogenesis that could potentially lead to osteolysis.

We demonstrate specific bioactivity of micropatterns towards osteogenesis, with more nodule formation and less osteoclastogenesis compared to planar controls. In addition, we found that that macrophage and osteoclast-like cells did not interact with the pits and formed fewer full-size osteoclast-like cells on the pitted surfaces.

This may have a role when designing ceramic orthopaedic implants. We are now using this co-culture for research on other micropatterned biomaterials.

78. POLY (ETHYL ACRYLATE) BRUSHES TO ENHANCE THE BIOLOGICAL ACTIVITY OF BIODEGRADABLE POLYMERS

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Poly L-Lactic acid (PLLA) has been used as a biodegradable polymer for many years; key characteristics of this polymer, its biocompatibility and controllable degradation within the body, make it a versatile and useful resource for regenerative medicine. One obstacle in utilising PLLA as a cellular environment for implantation is poor cellular adhesion due to its inefficient adsorption and expression of key biological signals. We aim to chemically modify PLLA surfaces with poly (ethyl acrylate) (PEA) brushes able to induce the organisation of the extracellular matrix component, fibronectin (FN), into physiological-like fibrils. These FN fibrils expose binding motifs critical for cell adhesion and differentiation, including domains for the binding of growth factors (GFs). With assembly of FN by PEA on the surface of a biodegradable polymer, we aim to create a more favourable and tuneable microenvironment for cells to adhere to and differentiate via controlled pathways through synergistic presentation of GFs.

The characteristic properties (stiffness, topology and biodegradation) of PLLA are ideal for bone regeneration applications *in vivo*, allowing stem cells to specialize within an implanted scaffold and eventually transfer the mechanical stress to the engineered tissue. We therefore aim to establish a protocol to polymerize PEA brushes on PLLA while maintaining the bulk properties of this polymer by using an activator regenerated electron transfer (ARGET) surface-initiated atomic transfer radical polymerisation (SI-ATRP) technique to achieve a thin molecular coating of PEA. Beside surface characterization via AFM, XPS and WCA to optimize PEA grafting, we investigated the biological activity of these surface modifications in terms of fibronectin adsorption and cell response. PEA brushes triggered FN organisation into fibrils, which retained their ability to specifically bind growth factors, particularly BMP2. Cell adhesion and differentiation studies confirmed the potential of PEA brushes to create controlled microenvironments via surface modification of a biodegradable polymer.

79. SYNTHETIC EXTRACELLULAR MATRICES FOR MAINTAINING STEM CELL PHENOTYPE

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Depriving stem cells of regulatory signals by isolating them from their niche leads to an uncontrolled phenotype *in vitro,* hallmarked by a loss of multipotency and a reduction in self-renewal capacity. However, segregating stem cells from their niche for culture is a critical step towards regenerative medicine but current approaches fail to supply the cues necessary to maintain stem cell phenotype. By combining surface chemistry and niche components we create several variations of a synthetic extracellular matrix (ECM) as systems to culture and maintain human bone marrow mesenchymal stem cells. To create these the archetypal bone marrow ECM protein fibronectin and growth factors VEGF and BMP2 are layered onto films of acrylate polymers poly(methyl-acrylate) or poly(ethyl-acrylate) in order to control their supramolecular organisation. Using In-Cell Western assays we demonstrate that the expression of osteogenic markers and MSC stemness markers are modulated after culture for 21 days in these systems. In particular, the combinations of poly(ethyl-acrylate) with fibronectin and growth factors increase expression of osteogenic markers, whereas the expression of stemness markers is increased on poly(methyl-acrylate) with fibronectin. Further, we investigate the effect of the different ECM combinations on cytoskeletal organisation and interaction using high-content imaging and automated fluorescence microscopy. This work was funded by the BBSRC WestBio DTP.

80. A VALIDATED SPECIMEN SPECIFIC FINITE ELEMENT MODEL OF VERTEBRAL BODY FAILURE

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Numerical models are widely used to evaluate the mechanical behaviour of vertebral bodies (VB) subject to different loading conditions. The validity of the vast majority of these is confined to the elastic region, and here good agreement with experimental data has been demonstrated. However this approach is poorly predictive of plastic failure. The present study aims to address this limitation and simulate the onset of yield. Six porcine VBs (from C2 to C7) were dissected from a spine specimen, potted in PMMA bone cement and Micro-CT imaged using a Nikon XT225 ST scanner (Nikon Metrology UK, Hertfordshire, UK). A compressive load was applied to each specimen with an Instron 5967, 30 kN materials testing machine (Instron, High Wycombe, UK) at a rate of 1000 N/min. Specimen-specific FE models of all specimens were created by segmenting and meshing the micro-CT images (ScanIP, Simpleware, UK), material properties were assigned from the grayscale value and the compression experiment was repeated in-silico. Conversion factors for the Young's modulus (kE), the Yield stress (ky), the Tangent (ktan) and the density (kρ) were determined for the grayscale values to minimise the error between experimental and numerical load-displacement behaviour. This allowed an excellent match between experiment and simulation results. The difference between experimental and numeric results for vertical displacement was typically 1% at 2000 N, between 1.5 and 3 % for 4000 N and between 2 and 3% for 5000 N, the latter typically representing the onset of yield. In this study, a technique allowing the prediction of the load-displacement behaviour of VBs subject to compression was developed. The novelty in the proposed approach rests with the fact that the onset of yield, crucial in determining subsequent failure modes, can also be modelled. This paves the way for more accurate FEA models aimed at predicting the failure modes of the spine.

81. COLLAGEN BECOMES ORDERED PRIOR TO MINERAL DEPOSITION

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The mechanism of the mineralisation of collagen is not fully established. Osteoporosis and osteoarthritis are debilitating bone diseases that are associated with a change in bone composition and bone mineralisation. The study and diagnosis of bone diseases are largely based on X-ray technologies but these methods do not provide information on the component of bone and therefore do not fully characterise this complex material and its role in bone strength. Raman spectroscopy however may be used non-invasively, and transcutaneously, to provide an overall biochemical signature.
Turkey leg tendons(TLTs) are recognised as a model organ for studying distinct regions of mineralised and non-mineralised collagen. The aim of this study is to test the hypothesis that Raman spectroscopy can be used to identify differences in the collagen secondary structure between regions of young TLTs that will remain non-mineralised and those that become mineralised.

Extensor tendons from six 'young' turkeys (11 weeks old; no mineralisation) and six 'mature' turkeys (18 weeks of age; distinct mineralised sections) were collected and spectra were acquired along each tendon using a Renishaw *inVia* (Renishaw plc, Gloucestershire, UK) Raman microscope (830 nm excitation wavelength).

Spectra across the transition zone of the mature TLTs and radiographs were used to confirm the presence/absence of mineral corresponding to the mature/young TLTs. Further Raman analysis revealed that as the phosphate(mineral) peak increased in height there was a change in the secondary structure of collagen, correlating to an increase in the Amid III:AmideI. Analysis of the young TLTs also revealed a change in AmideIII:AmideI along the length of the tendon and at the predicted transition zones.

The data confirm the hypothesis and establish that collagen becomes more ordered prior to mineralisation. These results may help identify the mechanisms controlling mineralisation and contribute to new treatments of bone diseases, where mineralisation has been disrupted.

82. INNOVATION TRENDS IN BONE ENGINEERING

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We gathered and analysed publicly available information from patents, clinical trials and registries from regulatory agencies related to the treatment of bone diseases with medical devices or advanced therapies. These are sources of information that are time and field specific and allow us to glimpse consecutive and critical frames of the translation of new scientific knowledge into market ready products.
We provide a perspective on current innovation risks and opportunities in bone biomedical engineering that may be used for early strategic decision making for organizations in the field. Trends and current approaches in the advancement of TERM products are discussed with particular attention to events in the development of cell therapies, the slow rise of orthobiologics during the last ten years and the current declining of growth factor related innovations.

83. A NOVEL TECHNIQUE FOR THE REPAIR OF MUSCLE TEARS: A COMBINATION OF SUTURES AND GLUE

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Muscle tears are very common injuries, especially in sport. While the vast majority of muscle tears can be successfully managed conservatively, surgery is required for severe muscle tears. Currently this surgical procedure uses sutures to bring the lacerated edges of the muscle together. There are two major problems with this technique: the strength of the repair and the presence of gapping. Our study sought to evaluate the use of a different muscle repair technique, a combination of sutures and glue.

To evaluate this novel method, the gastrocnemius muscles were dissected from twenty pre-culled Wister rats, one limb was assigned to the repair group and the contralateral limb used as a control. In each repair muscle, a full thickness mid-belly incision was made using a scalpel, before allocation to one of two repair methods: sutures alone and the combination of sutures and glue. Tension was then applied to each muscle by use of a mechanical testing machine and force and deflection were recorded. Furthermore, the presence and the force at which gapping between the repaired muscle appeared was observed.

The results revealed that a combination of sutures and glue is both significantly stronger at failure than the current repair method of sutures alone and the combined repair increases the resistance to gap formation in the muscle.
Although *in vivo* studies are required to assess the viability of glue in living tissue, these findings are potentially of great practical importance. They indicate that this novel combination of suture and glue would allow earlier mobilisation of the injured muscle, thereby leading to a quicker recovery.

84. LIVING BIOINTERFACES BASED ON NON-PATHOGENIC BACTERIA SUPPORT AND INDUCE STEM CELL DIFFERENTIATION.

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Lactococcus lactis is a lactic acid, non-pathogenic bacteria that has been genetically engineered to display the III_{7-10} fragment of the human fibronectin in its cell wall. This strain has been shown to develop biofilms on different abiotic surfaces, such as glass or synthetic polymers. In this work, this bacterial biofilm is used as a substrate for long-term mammalian cell culture (human bone marrow-derived mesenchymal stem cells, hMSCs) for up to 28 days. Both bacterial biofilm and mammalian cells showed good viability values at the end of the culture. The fibronectin fragment, which carries the adhesive RGD and the synergic PHSRN motifs induces cell adhesion on the hMSCs on its own, even when there is no other RGD sources in the medium or the substrate, to a degree that makes this engineered biofilm functionally equivalent to a substrate coated with 300 ng/cm^2 fibronectin.

In this work we explored the suitability of this substrate to induce osteogenic differentiation on the aforesaid hMSCs when supplementing the culture medium with bone morphogenetic protein-2 (BMP-2). The resulting cultures showed specific osteogenic differentiation markers such as osteocalcin expression, determined by immunofluorescence, and phosphate deposition, as determined by Von Kossa staining.

This biointerface based on living bacteria holds promise as a dynamic substrate for controlling stem cell fate and can be further engineered to express, on demand or in response to environmental conditions, other biochemical cues required to control stem cell differentiation.

85. REVIEW OF ETIOLOGY, DIAGNOSIS AND OUTCOMES FOLLOWING SEPSIS IN A NATIVE JOINT

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Suspected sepsis of a native joint poses a huge management challenge. There could be a number of conditions ranging from infective, reactive or inflammatory arthritis to crystal arthropathy. The incidence ranges from 4 to 29 per 100,000 person years. Rapid diagnosis and swift treatment is necessary to help prevent significant morbidity and mortality.

A retrospective analysis was undertaken to identify the etiology, causes and management of suspected septic arthritis. Case notes, microbiology results and prescription records were scrutinized. We excluded pediatric cases. All patients had a baseline serology followed by aseptic aspiration of the joint. Patients received appropriate antibiotics and joint debridement. Patients were followed up till satisfactory resolution of symptoms.

In 2014, 88 patients (age range 28–97 years) underwent a joint aspiration for suspected sepsis. Knee joint was the commonest joint aspirated in 49 (56%) patients. Organisms were identified in 15(17%) aspirates including Staphylococcus aureus (7), streptococci (5), E coli (2) and Streptococci Warneri (1). Three patients had history of steroid injection into the joint. All patients underwent surgical debridement and seven needed second washout. Organisms were identified in aspirate of three patients previously on antibiotics. Patients were prescribed one to ten weeks of appropriate antibiotics based on sensitivity. Flucloxacillin singly or in combination with Rifampicin was commonly used. 12 patients recovered and three patients died.

No organisms were isolated in 73 cases (26 cases aspirate positive for crystals). 35 patients received antibiotics and 11 joints debrided based on clinical suspicion. Remaining 38 patients did not receive any treatment. All patients made good recovery.

Management based on sound clinical judgment, prompt investigations, rapid surgical debridement and appropriate antibiotics can lead to satisfactory outcome.

86. AN INVESTIGATION OF HAPTIC MODELLING FOR MAXILLOFACIAL SURGICAL OPERATION TRAINING AND PLANNING

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This research investigates a haptic modelling approach where high resolutions are required for sensibility of force feedback in a target application - dental surgical operations. In particular the research focus is on maxillofacial deformity operations. The main aim of the research is to increase the realism of a computer model based simulation system that allows dental students and surgeons to feel like as if they were carrying out a real dental surgery procedure. A generic set of jaw bone models have been developed and validated by collaborating researchers from Glasgow Dental School. The model can be customized and obtained for each specific patient. A chosen model can be used to control the force feedback generated by a haptic device in order to give a realistic force feedback representation and experience for a user. This meets the requirements of the targeted dental operations. The simulation model is constructed based on force calculation model, which include the consideration of properties of bones, cutting tools used and position of cut. This haptical jaw bone model has been generalized based on a contact mechanic model Hertz's equation and is used as the driving haptical model for the study. Using a haptic device as a controller, a user can perform relevant operations, such as cutting procedure and manipulating bone segments from the virtual jaw bone model. The paper describes a frame work for a haptic assisted surgical plan (HASP) for maxilofacial deformity surgical planning. The haptic system generates the corresponding force feedback to a user as in a real world operation. It is planed that validation and feedback experiments will be conducted with dental students and surgeons to assess the effectiveness of the system in providing assistance for training of dental surgical procedures using the system.

87. Lower limb deformity is not a predictor of degenerative changes in the knee in early osteoarthritis

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A common orthopaedic presentation is knee pain with minimal or no radiographic evidence of osteoarthritis, most frequently in middle-aged patients considered young for joint replacement surgery. Standard knee radiograph grading methods are poor predictors of cartilage damage and therefore management decisions can prove difficult in these patients. This study explores mechanical alignment as a predictor of cartilage damage in the knees of patients awaiting arthroscopy.

This study analysed 105 hip-knee-ankle digital radiographs of painful knees awaiting arthroscopy (68 males, 37 females, mean age 50). Measurements of mechanical femorotibial alignment were made using centres of the femoral head, knee and ankle. Varus alignment was recorded as a negative angle and valgus as positive. Outerbridge grading (0-4) as recorded during arthroscopy was collected from patient casenotes. Medial and lateral compartment scores were calculated as the sum of grades (0-4) of the medial/lateral femoral condyle and medial/lateral tibial plateau respectively. Alignment and compartment scores were compared using Spearman's correlation coefficient, rho.

Mean alignment was -1.5° (SD 3.3, min -9.7°, max 7.8°). Overall there was a weak negative correlation between alignment and medial score, rho = -0.224 p=0.022 (more varus alignment indicating higher medial score) and a weak positive correlation between alignment and lateral score, rho = 0.204 p=0.037 (more valgus alignment indicating higher lateral scores). For males there was a weak negative correlation between alignment and medial score, rho = -0.299 p=0.013 and a trend to weak positive correlation between alignment and lateral score, rho = 0.232 p=0.057. No significant correlations were found for females.

All rho values in this study were between 0.2-0.3, indicating mechanical alignment can only explain 4-9% of the variation in degenerative changes. Therefore mechanical alignment was found not to be a reliable predictor of cartilage damage in this patient group.

88. PATIENT SATISFACTION WITH AN INNOVATIVE MULTIDISCIPLINARY PATHWAY IN THE MANAGEMENT OF CARPAL TUNNEL SYNDROME.

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Carpal tunnel syndrome (CTS) is a common orthopaedic referral requiring multiple clinic visits during treatment. This puts a huge strain on the availability of clinic slots and also delays timely treatment. We have introduced a unique multidisciplinary (Hand therapist led) pathway in the treatment of carpal tunnel syndrome to improve the efficiency of the service. The audit aims to assess patient satisfaction and safety with this new pathway in comparison with the traditional orthopaedic carpal tunnel pathway.

127 patients who had carpal tunnel decompression surgery over the past 12 months were contacted by telephone. They were grouped into two categories: 'Traditional pathway: reviewed initially by orthopaedic consultant' and 'New pathway: reviewed by occupational hand therapist (OHT)'. Patient satisfaction was assessed through a phone questionnaire.

All patients were happy with the care they received. 95% patients had good to excellent results following surgery. Out of 51 respondents, 20 patients were

reviewed by the OHT. 90% patients rated their overall experience as excellent. 100% of those reviewed by the OHT felt things were explained clearly and 100% did not mind being reviewed by the OHT.

The traditional pathway required approximately 3 orthopaedic consultations per patient. On a sessional basis, the cost of an OHT clinic is £50 compared to an orthopaedic consultant clinic £110. With the 20 patients reviewed by the OHT, we believe to have avoided 60 orthopaedic consultant clinic appointments. In view of the excellent patient satisfaction reviewed by the OHT. This is a suitable model to adopt across the National Health Service because it allows other orthopaedic clinics to take place simultaneously, delivers a safe, efficient and cost-effective service. However, it is only possible with efficient teamwork and patient education.

89. ***IN SITU* REMODELLING OF BIOMATERIALS: CULTURE PLATFORM FOR MESENCHYMAL STEM CELL SELF RENEWAL AND DIFFERENTIATION.**

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The maintenance of mesenchymal stem cells (MSC) *in vivo* directly relies on the dynamic and highly versatile nature of the extracellular matrix (ECM). This highly complex environment comprises many proteins and structures that translate any changes into strict genetic commitments that in turn control stem cell phenotype. Biomaterials approaches to replicate the ECM for stem cell culture have become common place. The innovative methods by which researchers employ materials (changes in surface chemistry, topography and stiffness) have expanded our knowledge on the nature of mesenchymal stem cell behaviour. However, these materials are only able to provide the cell with one behavioural cue: to self renew or differentiate. This work looks at harnessing the dynamic potential of solid phase peptide synthesis in an attempt to recreate the mesenchymal stem cell niche *in vitro*, and provide a surface in which we can spatiotemporally provide behavioural cues to cells. This technology holds the potential to maintain stem cell phenotype, as well as direct differentiation by manipulating the natural cellular enzyme secretion. Creating an enzyme responsive sequence that can be cleaved *in situ* allows cellular control over the material interface.

90. The clinical application of BMP for reconstruction of maxillary defect

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Orofacial clefts are one of the most common birth defects with an incidence of 1 in every 600-700 live births. Autogenous bone graft is the gold standard for the reconstruction however; it is associated with well documented morbidities.
The aim of the study was to assess the clinical application of BMP-7 for the reconstruction of alveolar cleft of the maxilla.

This study with conduct as prospective phase II clinical trial on 11 cases, 9 unilateral and two bilateral alveolar clefts. In all the cases the alveolar cleft was reconstructed with BMP-7 (Osigraft, OP1, Stryker Biotech, UK). For each case a vial of Osigraft® had 3.5mg of recombinant human osteogenic protein 1"Op-1" was applied at the alveolar defect. The graft was packed locally into the maxillary defect. Immediate post-operative radiographs were taken to assess the shape and appearance of the grafts. Six months following surgery, the patients underwent their final radiographic scan which was used for the assessment.

Clinically, postoperative complications were minimal; the surgical site healed well, all the patients were discharged from the hospital the next day after surgery. The extended of the bone formation in the unilateral alveolar cleft was scored as grade 1 (>75% in fill) in all the cases except one which scored grade II (50-70% bone infill).

In this study the donor site morbidity was completely eliminated by using rhMBP_7 for the reconstruction of the alveolar cleft. In addition, the duration of the surgical procedure was reduced and stay in the hospital was limited to one day. Radiographic assessment gave an objective and reliable indication on the quality and the quantity of the newly formed bone

91. HIP INJECTIONS: LOOKING FOR THE 'HALO'

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Early osteoarthritis (OA) can be treated with intra-articular steroid hip injections and delays the need for joint replacement. Prior to insertion, a radio-opaque dye is injected, a typical 'halo' sign develops at the head-neck interface and the position of the needle is confirmed using an image intensifier. These injections are administered for diagnostic or therapeutic purposes. The former confirms the source of pain is originating from the hip whilst patients with therapeutic indications avoid the need for other surgical interventions such as hip arthroplasty.

Retrospective analysis of all radio-opaque dye hip injections over the past eight months were analysed. These were grouped into two categories: diagnostic and therapeutic indications. Each radiograph was analysed to detect the classic 'halo' sign at the head-neck interface. The post-operative clinic appointment was reviewed for each patient to determine if pain was relieved and the reasons for any other investigations requested thereafter.

40 patients had a hip intra-articular steroid injection. 32 patients were classified as diagnostic whilst 8 were therapeutic. 30 patients had the classic 'halo'sign at the head-neck interface.13 patients from the diagnostic group felt their symptoms were relieved, but were also treated conservatively with physiotherapy or listed for a hip arthoplasty. For those patients in the diagnostic group who felt there was no change to their initial symptoms, were further investigated, commonly with a MRI to exclude back problems.

Patients commonly had temporary relief with hip injections, and those with OA were commonly listed for a hip arthroplasty in the following months. The patients whose radiographs did not show the typical contrast pattern, their post-clinic appointment confirmed their symptoms were never relieved. Thus, the 'halo' sign is a specific sign that confirms the appropriate site for needle positioning.

92. DEVELOPMENT OF A BESPOKE MOVEMENT ANALYSIS PROTOCOL FOR ROUTINE CLINICAL USE IN ORTHOPAEDICS

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Assessment of progress and outcome of orthopaedic interventions are often carried out using subjective observational methods. These may not be the most accurate or sensitive way to assess patient progress (Ong *et al.* 2008). In contrast, motion capture is currently the gold-standard for measuring human movement (Gage 1993; Cook *et al.* 2003) and the equipment cost is decreasing, making routine clinical use a possibility. However, current movement analysis protocols and setups are not suited for routine clinical use as they are time consuming and complex. Therefore, the aim of this study was to develop a protocol which could be easily adopted by the orthopaedic community and provide more sensitive outcome measures in routine clinical practice.

A bespoke, cluster based marker model (CM) was developed. Kinematics were calculated using the Grood and Suntay (1983) method and the kinematic output was compared to the current clinical gold-standard (Vicon Plug in Gait; PiG). Ten healthy volunteers wore a comprehensive marker set comprised of CM and PiG and performed 10 over-ground walking trials. Hip and knee flexion, abduction and rotation were compared along with ankle dorsi/plantar flexion. T-tests determined any significant difference between models.

The cluster based marker set was quick and easy to apply. When comparing the kinematic output between CM and PiG, there were some small but statistically significant differences. Differences were more likely to occur in rotations out with the sagittal plane.

CM provides a kinematic output comparable to that of the current clinical gold-standard. Differences in output may be due to different methods for estimating joint centres and calculating kinematics. In conclusion, CM is tailored for clinical use and should be considered the preferred option in routine clinical practice. Using the methods described, a gait test can be conducted in 10 minutes in the clinic by a physiotherapist or nurse.

93. A 3R'S REFINEMENT TO ASSESS ANALGESIA REGIMENS USING GAIT CHANGES IN MURINE SEPTIC ARTHRITIS THAT IS MORE ACCURATE THAN MOUSE GRIMACE SCORES

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Minimising the pain, suffering, distress or lasting harm to animals in scientific research is essential but the assessment of these parameters is often subjective. We compared two analgesia regimes using the mouse grimace score and gait changes in a murine model of septic arthritis.
Fourteen mice were inoculated with 10µL of 10^8 *S. aurues* suspended in PBS into the stifle joint. Seven mice received a single injection of buprenorphine on the day of inoculation only (Group A) whereas the other seven received daily injections (Group B). Gait was evaluated using the 'CatWalk' gait analysis system (Noldus Ltd.) at 0, 24 and 48 hours post inoculation. Prior to the gait analysis mice were video recorded for 5 minutes. Still images were developed and scored according to the mouse grimace score by five blinded, independent, expert assessors. Repeated measures analysis of variance with time and group as representative factors were performed. There was a statistically significant difference in the gait parameters between baseline measures and subsequent time points. The hind limb foot print size ($p = 0.040$), swing phase percentage ($p = 0.035$) and swing speed ($p = 0.025$) were statistically different between the 2 groups with Group A having features consistent with higher pain. Mouse grimace scores failed to show any difference between individual mice at different time points or between the groups as a whole.
We concluded that the optimal analgesia regimen is daily buprenorphine (Group B). Gait data was more sensitive than the grimace score for analysis of pain. Grimace scores were a poor method for analysis of pain that may have implications for their use as a single measure of pain in other studies. Our model could be used to assess the effectiveness of analgesia regimes for inflammatory pain that may be relevant to other experimental models.

94. INJECTABLE SCAFFOLD FOR THE RECONSTRUCTION OF CRITICAL-SIZE MANDIBULAR DEFECTS.

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The reconstruction of critical-size bone defects in the maxillofacial region following trauma or cancer treatment remains a challenge. The gold standard for the reconstruction of such defects is autogenous bone graft. This is because bone possesses the three properties required for bone regeneration; osteogenesis, osteoinduction and osteoconduction. But harvesting grafts results in significant patient morbidity. As a result several scaffolds have been developed to replace grafts. This project evaluated a novel injectable form of scaffold, together with Bone Morphogenetic Protein 7 (BMP-7) and mesenchymal stem cells (MSCs), in the regeneration of bone in critical-size mandibular defects in rabbits.

The scaffold construct was developed to induce bone formation within a pedicled muscle flap. A critical-size defect (20×15 mm^2) was created in the mandible of ten rabbits. The masseter muscle was adapted to fill the surgical defect and a combination of calcium sulphate/hydroxyapatite cement (CERAMENT™, SPINE SUPPORT), BMP-7 and MSCs was injected into the muscle. Bone regeneration was evaluated 3 months after surgery.

Limited areas of bone formation bridged the defect, despite new bone formation throughout the muscle and within the connective tissue. The bone was thicker in the bucco-lingual direction compared to the contra lateral (non-operated) side. Quantitative histomorphometric assessment showed that the average bone surface area was 21.2±6 mm^2, this was significantly greater than that of the contra-lateral side. The amounts of residual cement and soft tissue were 20±12% and 41±10%, respectively. The average mineral apposition rate (MAR) was 1.92 µm/day.

The findings demonstrated the potential use of local muscle flaps for injectable bio-cements loaded with BMP and seeded with MSCs to induce bone formation, but further development of this approach is required to improve the physical nature of the cement to allow a more comprehensive diffusion of the material within the muscle tissue.

95. UK VALIDATION OF THE FORGOTTEN JOINT SCORE-12 AS AN OUTCOME MEASURE FOR HIP AND KNEE ARTHROPLASTY

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The Forgotten Joint Score (FJS-12) is a recently developed outcome tool that assesses the patient's ability to forget about their artificial joint during everyday life. This has been suggested to offer a more sensitive outcome assessment, however detailed psychometric validation in a UK population is lacking.

Data were collected in a prospective cohort study of primary TKA and THA in a large UK orthopaedic teaching hospital. Patient outcomes were assessed with the FJS-12, Oxford Hip Score (OHS) Oxford Knee Score (OKS) at three time points: pre-surgery, 6 and 12 months post-operation. Internal consistency, validity, convergent validity, effect size for change over time, and ceiling effects were assessed.

Data from 231 TKA and 205 THA patients were available for analysis. The FJS-12 showed high internal consistency (Cronbach $\alpha = 0.97$ in TKAs, and 0.98 in THAs). Corrected item-total correlations indicated as well a high degree of internal consistency (r=0.70-0.89 in TKAs, and r=0.77-0.91 in THAs). Convergent validity analysis showed high correlations with the Oxford Scores (r=0.85 in TKAs and r =0.79 for THAs). Between 6- and 12-month follow-up change was higher for the FJS-12 than for the OHS in THA patients (effect size Cohen's d was 0.21 vs -0.03). In TKA patients the effect size for change in this period was 0.12 for the FJS-12 and 0.06 for the OKS. Ceiling effects at 1 year follow-up were low for the FJS-12 with 3.9% of TKA patients and 8.8% of THA patients obtaining the best possible score.

The FJS-12 demonstrates strong measurement properties in terms of validity, internal consistency and sensitivity to change in both hip and knee arthroplasty patients. Low ceiling effects and good relative validity suggest that this score may be beneficial for monitoring longer term arthroplasty outcomes, especially in high performing groups.

96. EVALUATION OF THE RESULTS OF TREATMENT OF PATELLAR FRACTURES BY CANNULATED SCREWS WITH THREADED TENSION BAND WIRE

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The patella is an important component of the extensor mechanism of the knee. Patellar fractures need to be fixed if displacement occurs more than 2 mm. Transverse fractures comprise the largest category. Several different techniques for internal fixation have been employed.

The aim of this work was to evaluate the results of treatment of transverse patellar fractures with figure of eight wiring through cannulated screws.

Twenty patients were included in the study,all suffering from displaced transverse patellar fractures.All were treated by open reduction and internal fixation with figure of eight tension band wire through 4.0 mm cannulated screws.

All patients were assessed after 1 month, 3 months and 6 months according to a modified Hospital for special surgery (HSS) knee scoring system.Because varus and valgus knee alignment and stability are not affected by patellar fracture fixation, the ten points assigned to these functions are eliminated, making the highest score ninety points.Excellent results are considered with points from 75 to 90, good from 60 to 74, fair from 50 to 59 and poor with points below 50.

The final results of the study showed fourteen patients (70%) had excellent results, five (25%) good result, one (5%) fair result and no patient had a poor result.There was a statistically significant improvement of the patients' score throughout the follow up period.

The complications occurred included knee pain in one patient (5%), loss of terminal flexion of knee occurred in three patients (15%), one patient lost 30 degrees, another lost 20 degrees while the last lost 10 degrees. There were no cases with extension lag in this series.

Treatment of patellar fractures using figure of eight wiring through cannulated screws is an easy technique which gives good stability leading to good results with a low complication rate.

97. IMPROVING THE ASSESSMENT AND DOCUMENTATION OF POST-OPERATIVE REVIEWS ON ELECTIVE ORTHOPAEDIC LOWER LIMB ARTHROPLASTY PATIENTS.

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The assessment of post-operative patients is vital to identify any clinical deviation from the normal clinical path. A large degree had been noted in the variability of reviews by junior doctors on the first post-operative day. Often foundation doctors who had little experience in orthopaedics were unaware of key components of the clinical examination which should be performed and documented. Good clinical record keeping is essential for effective continuity of care in the post-operative period. Our aim was therefore to improve and reduce variability of the care received by patients on day one post elective lower limb arthroplasty by the introduction of a review proforma.

A quantitative composite score comprising the 12 most important aspects for review and documentation was used retrospectively to assess patient's notes. A survey was sent to junior doctors as a qualitative measure. Data was collected before and after the introduction of the proforma to see whether it had improved the standard and consistency of patient reviews.

Before any intervention the average scores were 7.3/12 (THR[1], n=13) and 5.9 (TKR[2], N=20). After implementing the proforma the average score for THR was 8.3/12 (n=23) and 7/12 (n=23) for TKR. On average there was a 19% increase in score for THR and 22% increase in score for TKR. Qualitative studies showed that the proforma helped junior doctors feel significantly more confident in assessing patients.

This quality improvement project has developed a standardised review process for lower limb arthroplasty patients as well as improving confidence of junior doctors during their trauma and orthopaedics rotation. Future work will be the introduction of a teaching session for new rotating juniors and further improvements on the proforma with a view to implement it as a formal trust-certified document.

98. TRAFIC (TRAUMA ASSESSMENT AND FOLLOW UP WITH INTEGRATION OF MODERN COMMUNICATION DEVICES) – RESULTS OF A PILOT STUDY.

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7.5 million people are estimated to attend trauma and orthopaedic clinics in the UK per year. Compared to elective orthopaedics, collection of patient reported outcome measures (PROMs) in trauma clinics is relatively non-existent. This pilot study aimed to assess the feasibility of prospectively collecting PROMs using a web based APP downloaded onto tablet computers (TCs) in the trauma clinic setting.

Following local research ethics approval, a web-based APP (TRAFIC) was developed and downloaded onto TCs. It consisted of a total of 21 questions (demographic information, employment, and rehabilitation status as well as quality of life (EQ5D)). With the aid of a medical professional, eligible patients were invited to use TRAFIC during the 'lag period' from 'booking in' to 'being called' for their appointment. Total time spent using the app (APP-time) was recorded as well as the total time spent in the outpatient department (CLINIC-time).

Ninety-nine patients were recruited for the study n=49 males, n=50 females. All recruited patients completed all questionnaire items with a median APP-time of 5 min. (range 2-118 min). The median 'lag' period was 16 min. (range 2-166min.), with a median CLINIC-time of 81 min (range 4 – 428min.). Recruited patients were generally receptive to this method of collecting data.

PROMs collection in the trauma OPD using a web based APP on TCs is feasible (APP-time falls well within the 'lag period') and well tolerated by patients.

99. SURFACE MOBILITY OF ECM PROTEINS TO ENHANCE GROWTH FACTOR POTENCY

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The mobility, anchorage and conformation of extracellular matrix (ECM) ligands have been implicated as important modulators of cell behaviour. Major protein components of the ECM including fibronectin encompass cells *in vivo* and bear not only specific structural binding sites for interactions with cellular adhesion molecules such as integrins, but also contain promiscuous sites [3] which bind and synergistically present growth factors [4] to their corresponding cellular receptors. In essence, ECM protein coatings are being utilized *in vitro* as a tool to control cell fate by acting as a growth factor reservoir. This has been shown to be more efficient than the conventional approach of culturing cells in growth factor supplemented medium, as less of the growth factor is required. Here we use a unique set of four polymers – Poly(methyl-, ethyl-, butyl- and hexyl- acrylate), i.e. PMA, PEA, PBA and PHA – characterised by similar surface properties e.g. stiffness but with increasing molecular surface mobility, which we have shown to be translated to the protein layer at the material interface. Three of these polymers spontaneously

induce the assembly of physiological-like fibronectin nanonetworks [2]. We investigated the influence of polymer mobility on stem cell fate and have shown using ELISAs a greater exposure of fibronectin sites important for cell adhesion and differentiation in the FN network conformation compared to the globular conformation on PMA, thus producing a non-monotonic effect on differentiation of mouse pre-myoblasts [1]. Moreover, we show using ELISAs more BMP-2 growth factor is bound on PEA compared to the rest of the polymers. This correlates to our observation of increased osteogenic differentiation of human bone marrow mesenchymal stem cells (hMSCs - BM) grown on PEA compared to the rest of the polymers. We hypothesize that PEA is more efficient at exposing FN sites important for BMP-2 presentation.

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100. VITRONECTIN AS A FINE REGULATOR OF CELL RESPONSE IN FIBRILLAR FIBRONECTIN MICROENVIRONMENTS

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Surface functionalization strategies of synthetic materials for regenerative medicine applications, including bone tissue engineering, comprise the development of microenvironments that recapitulate the physical and biochemical cues of physiological extracellular matrices. In our group, we have established a robust route to engineer fibrillar fibronectin (FN) matrices upon adsorption of the protein onto a specific polymer surface (poly ethyl acrylate); these physiological-like FN fibrils have an enhanced biological activity due to the exposure of the central cell binding domain ($FNIII_{9\text{-}10}$) that promotes $\alpha_5\beta_1$ engagement and of a promiscuous growth factor (GF) binding domain ($FNIII_{13\text{-}14}$) that allows for integrin-GF synergistic signalling.

In this work, we aim to augment the complexity of this artificial fibrillar microenvironment by introducing another multi-functional adhesive glycoprotein, vitronectin (VN), which possesses both cell binding (through α_v integrins) and GF binding domains. By adsorbing mixtures of FN and VN in different ratios, we found that co-adsorption with as much as 50% of VN led to increased FN adsorption, improved fibril formation and enhanced VN exposure (cooperative effect). As a result, the addition of VN to the protein network altered both the physical and biochemical properties of the microenvironment. Indeed, incorporation of VN to the protein network facilitated the mobility of the protein at the material interface and the ability of cells to reorganise the adsorbed FN layer. Moreover, using an established cell line with differentiation potential (C2C12 myoblasts), the presence of VN was shown to non-monotonically tune the degree of cell differentiation whilst monotonically increasing the level of cell fusion, probably through an interplay of interface mobility and engagement of vitronectin receptors.

We have revealed that substrate-induced protein matrices resulting from the cooperative adsorption of FN and VN have the potential to fine-tune cell response; this could be further exploited to modulate synergistic signalling in the presence of bound GFs.

101. A NEW MODEL OF MURINE SEPTIC ARTHRITIS ESTABLISHED VIA DIRECT STIFLE JOINT INOCULATION

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Approximately 10,000 people in the UK develop septic arthritis each year. Septic arthritis is a destructive disease resulting in damage to articular cartilage and despite current treatment strategies up to 50% of patients affected develop worsening joint function. It is imperative that research is conducted to clarify the causes of damage so that chondroprotective treatments can be developed.

The majority of our current understanding has been elucidated using murine models where infection is established via the haematogenous injection of bacteria that then home to the joints. Using this model the number and location of infected joints cannot be controlled. We have shown that direct joint inoculation is the predominant route of infection in patients with healthy articular cartilage and it is possible that the disease pathogenesis in these patients is different to those infected haematogenously.

We developed a murine model of septic arthritis based on direct joint inoculation with *Staphylococcus aureus*, which is the most common infecting organism. Adult male C57Bl/6 mice are individually anaesthetised with 5% isofluorane before injection with 10µL of a 10^7 solution of *S. aureus* suspended in phosphate buffered saline into the stifle joint. Using this dose we were able to achieve infection in 100% of animals using wild type (8325-4) *S. aureus* along with many isogenic mutant strains that will allow the study of the effects of isolated *S. aureus* characteristics. There were no adverse effects when the animals were injected with PBS alone.

Our model offers unique advantages to the haematogenous model including the certainty of knowing when the joint infection begins. This has implications for monitoring time dependent changes such as chondrocyte viability. Limiting the infection to the stifle joint allows for accurate monitoring of subtle gait changes that may be useful in comparing different treatments in live animals.

102. STRUCTURAL AND CELLULAR CHANGES IN SUBCHONDRAL BONE IN OBESE OA PATIENTS.

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Osteoarthritis (OA) is a major debilitating joint disease and a leading cause for Total Knee Replacement (TKR) surgery. OA is well known as "wear and tear" disease with obesity being one of its main risk factors. However, more recent studies show that biomolecular processes are also important triggers for cartilage and bone damage. Adipose tissue is known to be a reservoir for immune cells such as macrophages which are potent producers of pro-inflammatory molecules that could be involved in the pathogenesis of OA.

In the present study, we collected 50 femoral condyles at the time of TKR from male and female patients chosen because they were lean or obese. For this study lean was defined as a BMI<25 and obese as a BMI>35. The samples were scanned using micro-Computed Tomography (µCT) and values obtained for Bone Mineral Density (BMD), Bone Volume/Total Volume (BV/TV), Trabecular Thickness (Tr.Th.), Trabecular Spacing (Tr.Sp.) and Trabecular Number (Tr.N.). In addition, 5 samples from each group were decalcified and paraffin-embedded. Then 5µm sections were stained with an anti-hCD68 antibody.

The data obtained demonstrated that the BMD and Trabecular Thickness (Tr.Th.) scores were significantly lower in the samples obtained from obese males compared to lean males. This difference was not seen in females. There was no difference in other µCT parameters. The immunochemical analysis demonstrated a significantly higher content of $CD68^+$ positive cells in the bone marrow of obese patients compared to lean ones.

The data obtained suggests that there are differences both at the structural and cellular levels in the subchondral bone of obese compared to lean OA patients which could potentially have a role in the pathogenesis or progression of OA.

103. ISOLATION OF LOW VOLUMES OF SILICON NITRIDE PARTICLES FROM TISSUE

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Adverse biological responses to wear debris generated by total hip replacements (THRs) limit the lifetime of such devices [1]. This has led to the development of biocompatible coatings for prostheses. Silicon nitride (SiN) coatings are highly wear resistant and any resultant wear debris are soluble, reducing the possibility of a chronic inflammatory reaction [2]. SiN wear debris produced from coatings have not been characterized *in vivo*. The aim of this research is to develop a sensitive method for isolating low volumes of SiN wear debris from periprosthetic tissue.

Commercial silicon nitride particles of <50nm (Sigma Aldrich) were incubated with formalin fixed sheep synovium at a volume of 0.01mm^3 /g of tissue (n=3). The tissue was digested with papain (1.56mg/ml) and proteinase K (1mg/ml) and samples were subjected to density gradient ultracentrifugation using sodium polytungstate (SPT) to remove protein from the particles [3]. Control tissue samples, to which no particles were added, were also subjected to the procedure. Particles were washed to remove residual SPT and filtered onto 15nm filters. The filtered particles were imaged by scanning electron microscopy and positively identified by elemental analysis before and after the isolation procedure. To validate whether the isolation method affected particle size or morphology, imaging software (imageJ) was used to determine size distributions and morphological parameters of the particles. A Kolmogorov-Smirnov test was used to statistically analyze the data.

The particle size distributions of isolated and non-isolated particles were similar. Morphology in terms of roundness and aspect ratio was unchanged by the procedure. Future work aims to test the method on titanium and cobalt chrome wear debris generated by a pin-on-plate wear simulator. The method will then be applied to isolate and characterise particles from *in vivo* studies of novel SiN coated prostheses in a rabbit and sheep model.

104. GENERATION OF A JOINT ANGLE-PASSIVE FORCE MODEL TO QUANTIFY DIFFERENCES BETWEEN SURGICAL INTERVENTIONS FOR TORN MUSCLE: A NOVEL EXPERIMENTAL TECHNIQUE

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Muscle tears are common and occasionally require surgical intervention. Currently, after surgical intervention for grade III tears, there is no method of predicting passive range of motion available to patients postoperatively before re-rupture. This project aimed to generate a joint angle-passive force model, which could be used to quantify predicted passive range of motion available after repair of grade III muscle tear.

Twenty pre-culled Wistar rat hindlimbs had tibialis anterior and gastrocnemius lengths measured at incremented femorotibial and talocrural angles. Both muscles were then isolated using a novel surgical technique, and a tensile force was applied by a Zwick-Roell z005 mechanical testing machine until failure. The force and increase in muscle length were recorded, allowing a talocrural angle-passive force model to be generated. This model was used to assess two surgical interventions for grade III muscle tears in a within-subjects, controlled trial. Primary outcomes of mean peak force per kilogram, and corresponding talocrural angle were used in this assessment.

Mean gastrocnemius length increased significantly as femorotibial angle increased ($p<0.01$). Inverse relationships between tibialis anteriors and gastrocnemii were identified regarding talocrural angle and muscle length, as well as talocrural angle and passive force. A joint angle-passive force model was generated to determine that: a significant difference between the two repair methods (71.4%; $p<0.001$) for tensile strength (peak force per kilogram) before re-rupture corresponded to a significant difference (81.1%; $p<0.001$) increase in dorsiflexion range before risk of re-rupture, using the talocrural angle-passive force model

A novel joint angle-passive force model was generated. This could be used to predict passive range of motion available to patients after repair of grade III muscle tears, before predicted re-rupture. This has implications in both research (when evaluating novel surgical interventions) and clinical practice (advising of suitable postoperative activities).

105. INSERTIONAL ANATOMY OF THE PERONEUS BREVIS TENDON

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The fracture of the metaphyseal-diaphyseal junction at the base of the 5th metatarsal is known as Jones fracture. One of the surgical managements of the Jones fracture is plating. The aim of this study was to evaluate insertional footprint of the peroneus brevis tendon (PBT) and to define “safe zone” for placement of the plate.

Forty one-formalin fixed cadaveric feet were dissected to evaluate insertional footprint of the PBT. After isolation of the peroneus brevis muscle, dissection was carried out along the tendon up to its bony insertions. Subsequently, insertional footprints were identified on the bone surface and marked with ink. Photographs were taken after each step of the footprinting process. To assess the PBT footprint at the base of the 5th metatarsal, following features were evaluated: area of insertion (AOI) (mm^2), length (mm), width (mm) and shape. All measurements were performed using Image J software.

The mean AOI of the PBT at the base of the 5th metatarsal was 54.49 ±16.46 mm^2, the length 15.98 ± 5.11 mm and the width 4.69 ± 1.39 mm. Analysis of the shapes of the AOI at the base of the 5th metatarsal revealed four different types: kidney (n=12), diamond (n=9), oval (n=7) and crescent (n=13). Eleven (26.8%) specimens showed evidence of additional bony insertions.

This anatomical study evaluated variations and insertional footprint of the PBT. Findings of this study can be used to identify “safe zone”, to prevent damage of the PBT, when placing the plate for fixation of the proximal 5th metatarsal fractures.

106. SHORT-TERM KNEE FLEXION DURING STAIR ASCENT IN TOTAL KNEE ARTHROPLASTY PATIENTS

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Stair ascent is a demanding activity which requires around 85° of knee flexion. Analysing this task may give an indication of Total Knee Arthroplasty (TKA) joint function. This study looked at short-term outcomes to give information regarding initial recovery after TKA surgery.

Three-dimensional motion analysis was carried out on five healthy control participants and five TKA patients (Columbus®, B. Braun Aesculap, Tuttlingen) performing five stair ascents at their own self-selected pace, choosing whether or not to use handrails. Control data were recorded at one assessment and patient data both pre-operatively and at mean follow up of 10 weeks (8 to 12) post-operatively. The maximum knee flexion achieved during stair ascent was calculated.

Four patients walked with a step over step strategy enabling comparison with the control group. There was no change in mean flexion angle from pre-operative to post-operation for either the operated side [mean pre-operatively=84° (76°-94°) vs. 82° (79°-86°) post-operatively , paired t-test p=0.67] or the non-operated side [mean pre-operatively=81° (61°-87°) vs. 81° (70°-95°) postoperatively, paired t-test p=0.56]. This was lower than mean for the control group, 97° (90°-106°) t-test p<0.001. The pre- and post-operative flexion angles of the patient who walked with a step by step strategy was 55° and 56° on the operated side and 43° and 52° on the non-operated side.

Knee flexion during stair ascent was similar both pre- and at 10 weeks post operation. Post-operative function did not reach control group values. The large variation between individuals for flexion of the non-operated side may represent different strategies for stair ascent: higher angles to achieve a greater ground clearance for safety, or lower angles to allow the patient to ascend faster so the operated support leg spends less time under load. Further work on a larger number of patients is required to understand this finding.

107. EVALUATION OF OPEN REDUCTION AND INTERNAL FIXATION USING THE DISTAL RADIAL VARIABLE ANGLE LOCKING PLATE

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Distal radial fractures are extremely common injuries. The optimal management of such fractures remains controversial. The goal of treatment is to reconstruct the radiocarpal and the radioulnar joints to restore normal kinematics and achieve optimal outcomes. Parameters used to assess reduction are radial inclination, radial shortening and volar tilt. Variable angle locking (VAL) plates maintain angular stability while allowing variation to the angle of the screws.

We included all patients that had VAL plating over a two year period from December 2012 to December 2014.The Frykman classification was used to classify the fractures. Fifty patients had been operated on and five patients were excluded as they were followed up at their local hospitals. Thirty-five patients (78%) had been discharged without any complications. Ten patients (22%) had complications. Six of these (13%) had complications that did not need any intervention and were discharged, as they were symptom free. Complications in this group consisted of transient median nerve symptoms, radial shortening, screw penetrating distal radioulnar joint and displacement of the dorsal fragment. Four patients (9%) had complications that needed surgical intervention. Complications in this group were loss of reduction and collapse with intra-articular screws, screws penetrating the joint, flexor pollicis longus tendon rupture and prominent distal screws irritating the extensor tendons. Interventions were in the form of removal of metalwork and tendon transfer.

We conclude that VAL plating increases the stability of fixation but attention has to be given to position and length of screws to reduce complications and re-operation rates.

108. MATERIAL-DRIVEN FIBRONECTIN ASSEMBLY AND GROWTH FACTOR PRESENTATION FOR BONE REGENERATION IN 3D.

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Biomaterials provide are an attractive approach to augment and repair bone tissue lost through trauma or disease. Growth factors also show promise for enhanced bone regeneration *in vitro*. However, *in vivo*, current approaches lead to many off target effects. Here we present a simple 3D polymer and growth factor based system that robustly promotes osteo-induction of mesenchymal stem cells (MSCs). Poly-ethyl acrylate (PEA) scaffolds support the self-organization of extra cellular matrix (ECM) protein fibronectin into physiological-like nano-networks upon adsorption, exposing critical binding domains for integrins and allowing tethering and presentation of growth factors. We demonstrate ultra-low doses of the growth factor BMP-2 can induce enhanced osteogenesis of MSCs using protein analysis, RT-PCR and imaging techniques, and that this simple approach effectively increases osteo- related signaling through both growth factor receptor activation and integrin driven signaling to direct stem cell differentiation.

109. THE EFFECT OF SURFACE ROUGHNESS ON BONE CEMENT ADHESION

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The introduction of robotic technology into orthopaedic surgery presents a novel approach to removing bone tissue with a burr head drill. The use of a burr head drill can remove bone tissue and can be used with all implant designs but leaves different surface roughness compared to manual bone cutting saws. We investigate mechanical properties based solely on the effect of surface roughness associated with the use of the burr head and manual saw to determine if the surface roughness affects tensile and shear pull-off strengths, and whether the cement has a preference of surface roughness for adhesion.

Artificial bone material was used to create tensile and shear test samples in which two parallel surfaces were created normal to (tensile) and parallel to (shear) the loading direction. Surfaces were prepared either using a 6mm burr (rough) or a standard band saw (smooth). Either medium or high viscosity bone cement was used to adhere the surfaces. Specimens were loaded, under tension (n=146) or shear (n=86) conditions, until failure. For the mixed group, it was noted which side the cement was originally applied to, and which surface failed. Specimens of pure cement were also created and tested in tension.

The results showed that viscosity did not affect the mechanical behaviour of the pure cement specimens, when adhered to two surfaces significant differences were apparent ($P < 0.05$). Surface roughness under tensile conditions does affect the mechanical properties with smooth surface outperforming the rough ($P < 0.05$) whereas surface roughness has no effect on mechanical properties under shear conditions. Nevertheless the cement adhered to the rough surface preferentially, with the smooth interface failing first.

110. PHYSICOCHEMICAL PROPERTIES REGULATION OF HYDROXYAPATITE-TRICALCIUM PHOSPHATE-COLLAGEN THREE-DIMENSIONAL HYDROGEL FOR NANOKICKING STIMULATION

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Nanovibration stimulation (nanokicking) can promote osteoblastogenesis of human mesenchymal stem cells (MSCs) seeded in three-dimensional collagen hydrogels. However, the cells contract the gels causing poor gel/petri dish contact and poor fidelity of nanokick. This study aims to tune physicochemical properties using 3D printed scaffolds, hydroxyapatite (HA) and tricalcium phosphate (TCP) as well as altering collagen concentration.

Selected STRO1 human MSCs seeded in rat tail collagen hydrogels were prepared. Ring scaffolds were engineered and implanted in the gels for physical adjustment. Gel contraction was then observed for 4 weeks. For chemical adjustment, hydroxyapatite and tricalcium phosphate were added into the collagen hydrogel by percentage of the desired volume of HA and TCP at 0%, 0.035%, 0.09%, 0.15% respectively. Gel contraction were observed for 4 weeks. Cell viability, proliferation and differentiation were studied by Alamar blue, live-dead staining and qPCR. Rheology and nanodisplacement were also studied.

Adding the ring scaffold and using HA-TCP in the gel can reduce gel contraction rate over 4 weeks. At 0%, 0.035%, 0.09% and 0.15% volume of HA-TCP in the collagen hydrogel, elastic modulus measurement were 150, 175, 200, 160 Pa respectively. Average gel displacement at 1,000 Hz of nanokicking stimulation were 33.66, 74.19, 36.19 and 35.26 nanometer. At 4 weeks without stimulation, percentage of alamar blue reduction were 33.78%, 22.28%, 22.69% and 24.87%. Live-dead staining showed no cell death and qPCR showed an up-regulation of osteopontin corresponding a down-regulation of PPARγ in the 0.09% of HA-TCP-collagen hydrogel.

Use of scaffolds and composites can reduce gel contraction over a 4 week culture. As well as better physicochemical properties, osteoblastic differentiation, and hydrogel viscoelastic modulus can be increased. This work was funded by the Royal Thai Government.

111. AUTOMATIC QUALITY CONTROL FOR POPULATION IMAGING

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Over recent decades, large-scale image datasets have been increasingly collected and employed in the medical research for a comprehensive characterization of age-related diseases. Despite strict imaging protocols to ensure consistent high-quality images in a large population, various incidental artefacts are inevitable. Detecting these artefacts requires an exhausting amount of expert manual labour, which is simply infeasible on large datasets, and thereby, automatic image quality control appears as an emerging challenge in the medical imaging community. Here, we propose a completely unsupervised and general purpose method to detect and localise artefacts in large-scale medical image datasets.

The proposed method works on image patches. The image patches are grouped into different clusters, where the centroid of each cluster is called a visual word. This is analogous to the representation of a text document with a collection of words. We introduce a new image representation approach based on an optimal coverage of images with the learned visual words. A dissimilarity score is then computed between each selected visual word and the corresponding image patch. We learn the statistical distribution of the dissimilarity score over the images in the dataset, and then artefacts are detected as outliers.

We have tested the proposed algorithm on 1300 hip DXA scans collected using a Hologic QDR4500 Acclaim densitometer (Hologic Inc., Bedford, MA, USA) during a previous pharmaceutical clinical trial. DXA is the standard imaging technique for bone densitometry that suffers from different artefact types. To evaluate the performance of the proposed method on this dataset, 300 scans were randomly selected and manually annotated. We have found that less than 10% of images are distorted with artefacts. The sensitivity and specificity of the proposed method in detecting these artefacts are 81.82% and 94.12%, respectively.

112. The role of voltage-gated L-type calcium channels in nanotopography induced mesenchymal stem cell osteogenic differentiation

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Mesenchymal stem cells (MSCs) hold great promise as an autologous cell source for bone repair. Nanotopographical cues can be used to induce MSCs osteogenic differentiation by focal adhesions formation and coordination with BMP2 signalling. Bone is calcium rich tissue, and voltage-gated L-type calcium channels (VGCCs) play a crucial role to maintain intracellular Ca^{2+} homeostasis that contributes to functional activities of osteoblasts. Our metabolomics data and other work also suggest calcium channels are involved in the regulation of BMP2 expression. Thus, we hypothesize that VGCCs maybe a new regulator in osteoinduction of MSCs promoted by nanotopography.

VGCCs are large transmembrane protein complexes, composed of α1, α2, β and δ subunits, to couple membrane depolarization for cellular Ca^{2+} entry. The α1 subunit is the site of Ca^{2+} influx, while α2 regulates α1 expression and depolarization. In this study, we examined VGCCα1 and α2 expression of MSCs cultured on near-square 50 (NSQ50) osteogenic nanotopography and found α2 has significant abundance in MSCs on NSQ50 compared to those on planar control. NSQ50 induced Ca^{2+} entry was observed by measuring intracellular [Ca^{2+}], which demonstrates MSCs intracellular [Ca^{2+}] is significantly increased on NSQ50. Moreover, using nifedipine (Nif), a VGCCα1 blocker to investigate the role of VGCCs in NSQ50 induced MSCs osteogenic differentiation, we found the osteogenic transcription factor p-RUNX2 was significant decreased by blocking VGCCα1.

Our work suggests VGCCs involved in nanotopography induced MSCs osteogenic differentiation by up-regulating VGCCα2 that promotes depolarization of α1. The increase in intracellular Ca^{2+} entry contributes to RUNX2 phosphorylation and results in MSCs osteogenic differentiation. We also speculate VGCCs may coordinate with integrins and Smad signalling which have been found playing crucial roles in NSQ50 induced MSCs osteogenesis, as Ca^{2+} entry can modulate gene expression and signalling phosphorylation, including focal adhesion kinase (FAK) and Smad.

113. MOLECULAR CONTROL OF MESENCHYMAL STEM CELLS

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Mesenchymal Stem Cells (MSCs) are multipotent with connective tissue lineage and vast therapeutic potential. Practical issues concerning supply, storage and transport of MSCs must be resolved before wider use is viable. The aims of this study were:a) Investigate: seeding density, growth factor(GF) and growth surface's (topography's) impact upon multipotency maintenance.
b) Optimise undifferentiated growth in vitro; establish lowest seeding density and c) Establish cryopreservation's effect on unisolated bone marrow MSCs. **Methods:**
1.
Topographies:petri,Planar(PL),Square(SQ).Combinedwith: 2 i. Seeding densities (50, 500, 1000 cells/cm) ii. GFs:(VEGF, BMP-2, control)
Fourweekculture
In-Cell Western: markers: Stro-1 (MSC), Osteocalcin (OCN)(osteoblast) and CellTag(unspecialised cell).
IncreasedStro-1:CellTagratio=increasedmultipotentMSCs.
Preliminaryanalysisofunisolated,cryopreservedcultures.
Comparisonofcryopreservedanduncryopreservedcultures.

DAPI and phalloidin staining.
a. HighestStro-1:Celltag- 2 i. 50 cells/cm + PL ii. VEGF + SQ 2. FewMSCssurvivedcryopreservation.Nocellsculturedfromdonor1,donor2 grew mixed cell population. Multipotency retention greatest when combining: lower densities on planar surfaces or VEGF and square patterned surfaces. Further experiments investigating optimum conditions are indicated. However, at this early stage VEGF has emerged as a new method to maintain MSC clonogenicity.
It's encouraging that MSCs survive freezing before isolation, but method must be optimised to be clinically relevant. It is worth investigating this further due to its potential impact on use of MSCs in research and treatment.

114. Investigating the potential of periosteal cells for critical size bone defect repair

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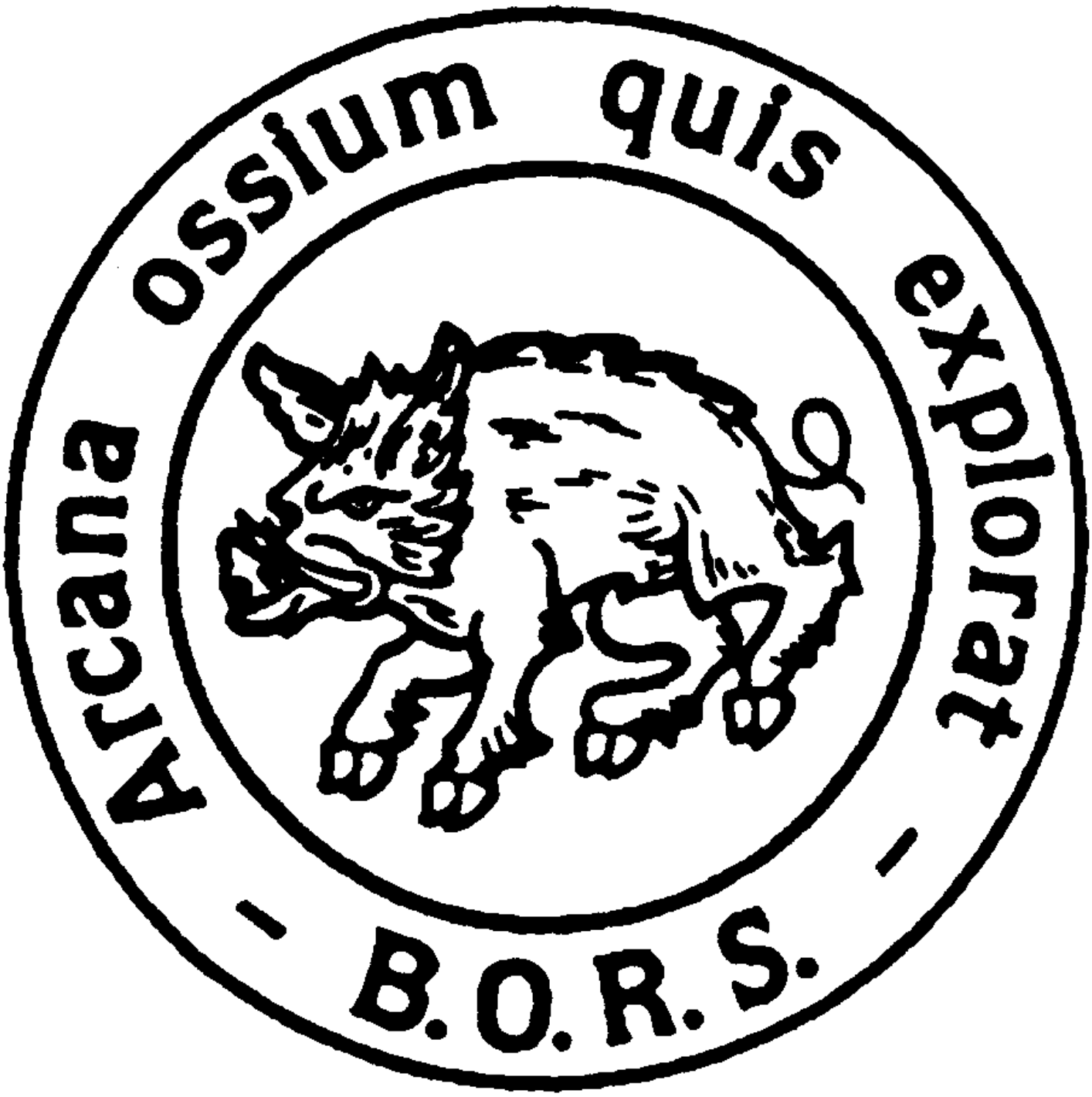
Surgical interventions for critical size bone defects remain suboptimal. Autologous bone grafting is the 'gold standard' procedure, however, risks insufficient graft volume and donor site morbidity. A tissue engineered 'hybrid graft' in which autologous mesenchymal stem cells (MSC) are combined with a bone scaffold and contained to a defect site by a barrier membrane is a new approach (1). MSCs populating such grafts *in vivo* could be of bone marrow (BM) or periosteal (P) origin. The aim of this study was to compare *in vitro* characteristics of human donor-matched periosteal MSCs (P-MSC) and BM-MSC in 2D culture prior to investigating their functionality on scaffolds.
Matched BM and long bone P were harvested from four healthy male donors (age range 35-61) undergoing trauma orthopaedic surgery. Cultures were established from $5.0x10^6$ BM nucleated cells or $1.5x10^5$ collagenase digested P nucleated cells. P-MSCs, measured as colony-forming unit-fibroblast, had considerably higher frequency compared to BM-MSC (average 5200 versus 6 MSC per $1x10^6$ nucleated cells, respectively). Similar to BM cultures, early-passage adherent P cultures had a classic MSC phenotype (positive for CD105, CD73 and CD90 and negative for CD45, CD14 and CD34) (2). In long-term culture, P-MSC continued to divide up to 20 population doublings (PDs) with faster growth rates up to the first passage (1.8 days/PD) compared to later passages (3.8 days/PD). Donor-matched BM-MSC had relatively fast growth rates up to the first passage (2.6 days/PD) after which growth plateaued. In order to identify potential *in vivo* markers of P-MSC, three extra MSC markers (MSCA-1, SUSD2 and CD271) (3,4) were tested showing P-MSC positivity for SUSD2 and the lack of MSCA-1 and CD271 expression, similar to BM-MSC. In conclusion, long bone P-MSCs had greater proliferative potential than donor-matched BM-MSCs, suggestive this MSC source merits further investigation in 3D conditions on scaffolds.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Transform Orthopaedics with Mako Today.

Stryker acquired MAKO Surgical Corporation in December 2013 with the goal of transforming the orthopaedic industry. The acquisition combines Stryker's market-leading implants enabled by MAKO's proprietary robotic-arm assisted technology. The current Mako System offers Partial Knee and Total Hip applications.