3rd Joint Meeting

of the

Bone Research Society

and the

British Orthopaedic Research Society

27-29 June 2011

Cambridge, UK

Final Programme and Abstracts

Bone Research Society

www.brsoc.org.uk

The Society (formerly known as the Bone and Tooth Society) is the oldest and largest scientific society in Europe that is dedicated to further research into clinical and basic science problems related to mineralised tissues. The meeting attracts a wide audience from throughout the UK and, increasingly, from continental Europe and further afield. The presentations are traditionally a balance between clinical and laboratory-based studies. The participation of young scientists and clinicians is actively encouraged.

Committee 2011
President: Jonathan Tobias (Bristol)
President Elect: Tim Arnett (London)
Secretary: Eugene McCloskey (Sheffield)
Treasurer: Nigel Loveridge (Cambridge)

Jacqueline Berry (Manchester)
Allie Gartland (Sheffield)
Celia Gregson (Bristol)
Vicky MacRae (Edinburgh)
Deborah Mason (Cardiff)
Isabel Orriss (London)
Sanjeev Patel (London
Ken Poole (Cambridge)

Membership Enquiries
Eugene McCloskey
Email: e.v.mccloskey@sheffield.ac.uk

Next Year's BRS Meeting
Manchester, 1-4 July 2012
Jointly with the National Osteoporosis Society and Paget's Association

British Orthopaedic Research Society

www.borsoc.org.uk

The British Orthopaedic Research Society (BORS) is a multidisciplinary association founded in 1961 and devoted to pursuing research relevant to orthopaedic and musculoskeletal surgery. The research interests of its membership (currently over 700) are varied and include:

Biological Science
Biomechanics
Osteo-articular Pathology
Biotribology
Molecular Biology
Bioengineering
Medical Imaging
Patient Management

Committee 2011
President: Allen Goodship (London)
Secretary: Gordon Blunn (London)
Treasurer: Xuebin Yang (Leeds)

Mark Birch (Newcastle upon Tyne)
Ben Bolland (Southampton)
Bruce Caterson (Cardiff
Mark Gaston (Edinburgh)
Richie Gill (Oxford)
Wasim Khan (London)
Iain McNamara (Cambridge)
Tony Miles (Bath)
Brigitte Scammell (Nottingham)
Hamish Simpson (Edinburgh)

Membership Enquiries
Tel: 0115 8231115
Fax: 0115 8231118
Email: oas-admin@nottingham.ac.uk

Next BORS Meeting
See website for details

Contents

The text of the abstracts is reproduced as submitted. Any tables and figures will be included with the published version, which for BORS will be the *Journal of Bone and Joint Surgery (Br)* and for BRS the online journal *Frontiers in Bone Endocrinology*.

The opinions and views expressed are those of the authors and have not been verified by the meeting Organisers or by the Societies, who accept no responsibility for the statements made or the accuracy of the data presented.

Supporters

The Bone Research Society and the British Orthopaedic Research Society are extremely grateful to the following companies who have agreed to support the meeting.

Further information about the exhibiting companies is given at the end of this book.

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Outline Programme

All sessions in West Road Concert Hall unless otherwise indicated

Monday 27 June

10:00	Registration/Coffee/Poster hanging
10:40	Welcome
10.45	Keynote Lecture
11:15	Symposium 1 **The Bone and Joint Decade: Closed Chapter or Open Book?**
12:30	Lunch and Posters (odd-numbered boards)
13:45	Symposium 2 **The 4Ms: Materials, Mineralisation, Microdamage and Mechanics**
15:20	Tea and Posters
16:15	Oral Communications
17:15	New Investigators' Session **Career pathways - examples and discussions**
18:30	End
19:30-23:00	Quiz Night/Buffet Supper Murray Edwards Hall

Tuesday 28 June

08:30	Satellite 1 **New Insights in the Treatment of Postmenopausal Osteoporosis** Supported by Servier *West Road Concert Hall*	
09:30	Clinical Update 1 *Divinity Lecture Theatre* (to 10:20)	Oral Communications (basic science) *West Road Concert Hall* (to 10:30)
10:20	Coffee and Posters (even-numbered boards)	
11.15	Symposium 3 **Cell-Cell Communication in Aged Skeletal Elements** *West Road Concert Hall*	
12.30	Lunch and Posters (even-numbered boards)	
13:30	Clinical Update 2 *Divinity Lecture Theatre* (to 14:45)	Oral Communications (basic science) *West Road Concert Hall* (to 14:50
14:45	Break	
15:00	Symposium 4 **Obesity, Energy Metabolism and Bone** *West Road Concert Hall*	Symposium 5 **Bone Graft Substitutes** *Divinity Lecture Theatre*
16:15	Tea and Posters (even-numbered boards)	
16:45	Clinical cases and clinical research *Divinity Lecture Theatre* (to 17:35)	Oral Communications (basic) *West Road Concert Hall*
17:45	Satellite 2 **A Force Against Fracture: New Perspectives in Preventing Bone Loss** Supported by Amgen *West Road Concert Hall*	
18:45	End	
19:45-22:30	Conference Dinner Cripps Hall, Queen's College	

Wednesday 29 June

09:00	Oral Communications BRS *Divinity Lecture Theatre*	Oral Communications BORS *West Road Concert Hall*
10:10	Coffee and Posters	
11:00	BRS Dent Lecture *West Road Concert Hall*	
11:30	Symposium 6 **Joint Development and Disease** *West Road Concert Hall*	
12:45	Lunch and posters	
13:40	BRS AGM *Divinity Lecture Theatre*	BORS AGM *West Road Concert Hall*
14:00	Oral Communications BRS *Divinity Lecture Theatre*	Oral Communications BORS *West Road Concert Hall*
15:00	Oral Posters *West Road Concert Hall*	
16:00	Awards *West Road Concert Hall*	
16 :15	End of meeting	
	Buses to Cambridge Station	

Programme

All sessions in West Road Concert Hall unless otherwise indicated

Monday 27th June

10:00 Registration and coffee

Poster hanging

10:40 Welcome
Nigel Loveridge (Cambridge, UK)

10:45 Keynote Lecture
Chairs: Juliet Compston (Cambridge, UK) & Allen Goodship (London)

Sir Michael Berridge

11:15 Symposium 1
The Bone and Joint Decade: Closed Chapter or Open Book?

Chairs: David Marsh (London, UK) & Eugene McCloskey (Sheffield, UK)

11:15 **Nigel Arden** (Southampton, UK): Osteoarthritis

11:35 **John Kanis** (Sheffield, UK): Osteoporosis

11:55 **Keith Willett** (Oxford, UK): Trauma

12:15 Panel discussion

12:30 Lunch and Posters (odd-numbered boards)

13:45 Symposium 2
The 4Ms: Materials, Mineralisation, Microdamage and Mechanics

Chairs: Nigel Loveridge (Cambridge, UK) & Andrew McCaskie (Newcastle, UK)

13:45 **Chris Burgoyne** (Cambridge, UK): An Engineer's view

14:05 **David Burr** (Indianapolis, USA): Bone microdamage

14:25 **Peter Fratzl** (Potsdam, Germany): Bone materials

14:45 **John Currey** (York, UK): A Biologist's view

15:05 Panel discussion

15:20 Tea and Posters (odd-numbered boards)

16:15 **Oral Communications**
Chairs: Tim Arnett (London, UK) & Xuebin Yang (Leeds, UK)

16:15 OC1
OSTEOCYTES PROMOTE OSTEOBLAST PROLIFERATION DIRECTLY AND EXERT A DISTINCT EFFECT WITH MECHANCIAL STRAIN
RFL Suswillo, SCF Rawlinson*, GP Dowthwaite, LE Lanyon, AA Pitsillides
London

16:25 OC2
ELEVATED SCLEROSTIN LEVELS IN INDIVIDUALS WITH HIGH BONE MASS
CL Gregson*, KES Poole, SA Steel, J Ayuk, EV McCloskey, WD Fraser, JH Tobias
Bristol; Cambridge; Hull; Birmingham; Sheffield; Liverpool

16:35 OC3
MARKED REDUCTION IN THE EXPRESSION OF SCLEROSTIN IN OSTEOCYTES CLOSE TO THE SITE OF FEMORAL NECK FRACTURES
J Power, M Parker S Papapoulos, R van Bezooijen, J Reeve, N Loveridge*
Cambridge; Peterborough; Leiden

16:45 OC4
RESULTS OF BONE MORPHOGENETIC PROTEIN (BMP-7) ALONE OR IN COMBINATION WITH AUTOLOGOUS BONE GRAFT (ABG) FOR THE TREATMENT OF LONG BONE FRACTURE NON-UNIONS
A Shoaib*, MA Rashid, O Lahoti, AFG Groom, SL Phillips
London

16:55 OC5
EFFECTS OF CHRONIC SYSTEMIC METAL EXPOSURE ON WHOLE BODY BONE MINERAL DENSITY AND BONE TURNOVER IN THE LATE PERIOD AFTER METAL-ON-METAL HIP RESURFACING
J Prentice*, M Clark, I Stockley, JM Wilkinson
Sheffield

17:05 OC6
HIGH FAILURE RATES WITH A LARGE DIAMETER HYBRID METAL ON METAL TOTAL HIP REPLACEMENT: CLINICAL, RADIOLOGICAL AND RETRIEVAL ANALYSIS
BJRF Bolland*, DJ Culliford, DJ Langton, JPS Millington, NK Arden, JM Latham
Southampton; Newcastle; Oxford

17:15 New Investigators' Session
Career pathways - examples and discussions
This short informal session is designed for both non-clinical and clinical PhD students and Post-docs in their early research career.

We are inviting 3 speakers who have established careers and who have taken different career paths; one lab-based scientist, one clinical scientist, and one clinician researcher. Each will speak for 15 minutes outlining their career path to date, identifying the key decisions which they have made along the way - the good decisions and the not so good decisions (if any!), and reflect upon if they were to embark upon the same career now, how the system might have changed and how they might negotiate it now? After the 3 presentations we will open the session up to the floor for an informal panel discussion.

Speakers:

Nicola Crabtree (Birmingham, UK)
Nick Harvey (Southampton, UK)
Helen Knowles (Oxford, UK)

18:30 End

19:30- 23:00 Quiz Night/Buffet Supper
Murray Edwards Hall

Tuesday 28th June

08:30 **Satellite 1**
Supported by Servier

New Insights in the Treatment of Postmenopausal Osteoporosis

West Road Concert Hall

Chair: Juliet Compston (Cambridge, UK)

Eugene McCloskey (Sheffield UK): How does bone metabolism change with age and osteoporosis?

Juliet Compston (Cambridge, UK): What is the impact of our treatment options on bone metabolism? – New insights

Serge Ferrari (Geneva, Switzerland): What are the clinical implications in the fight against fractures?

09:30 **Clinical Update 1**

Divinity Lecture Theatre

(concurrent with Oral Communications, below)

Chairs: Ken Poole (Cambridge, UK) & Hamish Simpson (Edinburgh, UK)

09:30 **Juliet Compston** (Cambridge, UK): Bisphosphonates and atypical femur fractures: tip of the iceberg?

09:50 **Richard Keen** (London, UK): Preventing the second hip fracture; what drugs to give and when?

10:10 Panel discussion

10:20 Coffee and Posters (even-numbered boards)

09:30 **Oral Communications (basic science)**

West Road Concert Hall

(concurrent with Clinical Update, above)

Chairs: Alison Gartland (Sheffield, UK) & Tom Joyce (Newcastle upon Tyne, UK)

09:30 OC7
A BMPR1A SOLUBLE RECEPTOR CONSTRUCT INCREASES TIBIAL AND TRABECULAR BONE VOLUME IN VIVO
M Baud'huin*, D Lath, AD Chantry, J Seehra, RS Pearsall, I Bellantuono, PI Croucher
Sheffield, UK; Cambridge MA, USA

09:40 OC8
THE MOLECULAR MECHANISM OF ISCHAEMIC PRECONDITIONING IN SKELETAL MUSCLE CELLS
C McGuire*, P Walsh, KJ Mulhall
Dublin

09:50 OC9
ACCELERATED SKELETAL DEVELOPMENT AND HIGH BONE MASS IN MICE LACKING RAMP3
S Pacharne*, G Richards, K Caron and T Skerry
Sheffield, UK; Chapel Hill, USA

10:00 OC10
PAGET'S DISEASE-CAUSING MUTATIONS IN SEQUESTOSOME-1 AFFECT AUTOPHAGIC PROTEIN DEGRADATION
E Azzam*, A Duthie, MH Helfrich, LJ Hocking
Aberdeen

10:10 OC11
LACK OF NUCLEOTIDE PYROPHOSPHATASE PHOSPHODIESTERASE1 ACTIVITY IN Enpp1-/- MICE RESULTS IN ALTERED BONE DEVELOPMENT AND PATHOLOGICAL MINERALISATION OF SOFT TISSUE
NCW Mackenzie*, D Zhu, EM Milne , JL Millan, C Farquharson, VE MacRae
Edinburgh, UK; La Jolla, USA

10:20 OC12
METAL ION LEVELS AND CHROMOSOME ABERRATIONS IN METAL-ON-METAL (MoM) AND METAL-ON-POLYETHYLENE (MoP) TOTAL HIP ARTHROPLASTY
BS Dhinsa, JR Perera*, KR Gallagher, B Spiegelberg, S Hanna, S Tai, R Pollock, R Carrington, SR Cannon, TWR Briggs
Stanmore

10:30 Coffee and Posters (even-numbered boards)

11:15 Symposium 3
Cell-Cell Communication in Aged Skeletal Elements

West Road Concert Hall

Chairs: Colin Farquharson (Edinburgh, UK) & Tim Skerry (Sheffield, UK)

11:15 **James Edwards** (Oxford, UK): Ageing mechanisms in musculoskeletal biology

11:35 **Jean-Marie Delaisse** (Vejle, Denmark): Bone remodelling: how are bone resorption and formation integrated in a joint bone renewal program?

11:55 **Graham Russell** (Oxford/Sheffield, UK): Pharmacological targets in ageing bone

12:15 Panel discussion

12:30 Lunch and Posters (even-numbered boards)

13:30 **Clinical Update 2**

Divinity Lecture Theatre

(concurrent with Oral Communications, below)

Chairs: Ken Poole (Cambridge, UK) & Tim Briggs (London, UK)

13:30 **Patrick Case** (Bristol, UK):
The current understanding of the problems associated with metal-on-metal hip replacements with special reference to ALVAL

13:50 **Nick Higgins** (Cambridge, UK):
Vertebro/Kyphoplasty

14:10 **Richard Villar** (Cambridge, UK):
Femoroacetabular impingement: Is it important?

14:30 Panel discussion (to 14:45)

13:30 **Oral Communications (basic science)**

West Road Concert Hall

(concurrent with Clinical Update, above)

Chairs: Vicky McRae (Edinburgh, UK) & Richard Oreffo (Southampton, UK)

13:30 OC13
PLATELET RICH PLASMA IN ACCELERATED ACHILLES TENDON REGENERATION: A RANDOMIZED CONTROLLED TRIAL
J Alsousou*, R Handley, P Hulley, M Thompson, E McNally, P Harrison, K Willett
Oxford

13:40 OC14
ENRICHMENT OF SKELETAL STEM CELLS FROM BONE MARROW TO ENHANCE SKELETAL REGENERATION - A NOVEL CLINICAL TECHNIQUE
JO Smith*, JI Dawson, A Aarvold, AMH Jones, JN Ridgway, SJ Curran, DG Dunlop, ROC Oreffo
Southampton; York

13:50 OC15
THE SYSTEMIC STIMULATION OF MESENCHYMAL STEM CELLS (MSCS) IN BONE MARROW IN RESPONSE TO TRAUMA
HB Tan*, E Jones, L Kozera, K Henshaw, D McGonagle, PV Giannoudis
Leeds

14:00 OC16
HOW DO OSTEOCLASTS GENERATE ENERGY TO MAINTAIN BONE RESORPTION WITHIN A HYPOXIC ENVIRONMENT?
HJ Knowles*, NA Athanasou
Oxford

14:10 OC17
FLUID FLOW STIMULATES ATP RELEASE FROM HUMAN DERIVED OSTEOCLASTS WITHOUT CHANGING RESORPTION
RMH Rumney*, A Agrawal, K Shah, A Gartland
Sheffield

14:20 OC18
OSTEOBLAST EXTRACELLULAR MATRIX MINERALIZATION IS PROMOTED BY PHOSPHO1 OVEREXPRESSION
C Huesa*, M Yadav, E Seawright, JL Millan, C Farquharson
Edinburgh, UK; La Jolla, USA

14:30 OC19
IN VIVO EVALUATION OF MINERALISED COLLAGEN-GLYCOSAMINOGLYCAN AS A BONE GRAFT SUBSTITUTE
MQ Arumugam*, AK Lynn, N Rushton, RA Brooks
Cambridge

14:40 OC20
INTRAOSSEOUS TRANSCUTANEOUS AMPUTATION PROSTHESES VS DENTAL IMPLANTS: A COMPARISON BETWEEN KERATINOCYTE AND GINGIVAL EPITHELIAL CELL ADHESION IN VITRO
CJ Pendegrass*, C Fontaine, GW Blunn
Stanmore

14:50 Break

15:00 Symposium 4
Obesity, Energy Metabolism and Bone

West Road Concert Hall

(concurrent with Symposium 5, below)

Chairs: Celia Gregson (Bristol, UK) & Graham Russell (Oxford/Sheffield, UK)

15:00 **Antonio Vidal-Puig** (Cambridge, UK): Adipocytes and energy metabolism

15:20 **Chantal Chenu** (London, UK): Energy metabolism and bone

15:40 **Jon Tobias** (Bristol, UK): Obesity and bone in childhood

16:00 Panel discussion

16:15 Tea and Posters (even-numbered boards)

15:00 Symposium 5
Bone Graft Substitutes

Divinity Lecture Theatre

(concurrent with Symposium 4, above)

Chairs: Neil Rushton (Cambridge, UK) & Brigitte Scammell (Nottingham, UK)

15:00 **Gordon Blunn** (London, UK): Bone graft materials: osteoconduction and osteoinduction

15:20 **Molly Stevens** (London, UK): New materials-based strategies for bone repair

15:40 **Roger Brooks** (Cambridge, UK): What is the best source of cells for bone grafts?

16:00 Panel discussion

16:15 Tea and Posters (even-numbered boards)

16:45 **Clinical Cases and Research**

Divinity Lecture Theatre

(concurrent with Oral Communications, below)

Chairs: Cathy Holt (Cardiff, UK) & Sanjeev Patel (London, UK)

Clinical Research

16:45 OC21
SEPTIC ARTHRITIS VS TRANSIENT SYNOVITIS IN CHILDREN: A TERTIARY HEALTH CARE CENTRE STUDY
R Singhal*, D Perry, FN Khan, D Cohen, HL Stevenson, LA James, JS Sampath, CE Bruce
Macclesfield; Alder Hey, UK; Sparsh, India

16:55 OC22
HISTOLOGICAL RESULTS OF 406 BIOPSIES FOLLOWING ACI/MACI PROCEDURES FOR OSTEOCHONDRAL DEFECTS IN THE KNEE
AS Shekkeris*, JR Perera, G Bentley, AM Flanagan, J Miles, RWJ Carrington, JA Skinner, TWR Briggs
Stanmore

Clinical cases

17:05 CC1
FIBROBLAST GROWTH FACTOR-23: STRONG ASSOCIATION WITH THE RENAL FANCONI SYNDROME IN A PATIENT WITH ONCOGENIC OSTEOMALACIA
AGW Norden*, RJC Laing, RJ Unwin, OM Wrong, AJ Crisp
Cambridge; London

17:20 CC2
CORD COMPRESSION RESULTING FROM FIBROUS DYSPLASIA (FD) OF THE SPINE : A CASE REPORT
I Scott*, J Lucas, G Gnanasegaran, C Silve, I Fogelman, G Hampson
London, UK; Paris, France

17:35 Break

16:45 **Oral Communications (basic science)**

West Road Concert Hall

(concurrent with Clinical Cases and Research, above)

Chairs: Debbie Mason (Cardiff, UK) & Gordon Blunn (London, UK)

16:45 OC23
INVESTIGATING THE INTERACTION OF RAT OSTEOBLASTS WITH TITANIUM OXIDE NANOLAYERS
SR Deshmukh*, MA Birch, D Robbins, AW McCaskie
Newcastle upon Tyne

16:55 OC24
MEPE REGULATES GROWTH PLATE MINERALISATION THROUGH ITS CLEAVAGE TO THE ASARM PEPTIDE
KA Staines*, VE MacRae, C Farquharson
Edinburgh

17:05 OC25
THE EFFECT OF RECOMBINANT HUMAN FIBROBLAST GROWTH FACTOR-18 ON ARTICULAR CARTILAGE FOLLOWING SINGLE IMPACT LOAD
LV Barr*, FMD Henson , A Getgood , N Rushton
Cambridge

17:15 OC26
THE APPEARANCE AND MODULATION OF OSTEOCYTE MARKER EXPRESSION DURING CALCIFICATION OF VASCULAR SMOOTH MUSCLE CELLS
D Zhu*, NCW Mackenzie, JL Millán, C Farquharson, VE MacRae
Edinburgh, UK; Sanford, USA

17:25 OC27
DOES A STRONTIUM SUBSTITUTED BIOGLASS ENHANCE THE RATE OF BONE INGROWTH INTO A CRITICAL SIZE DEFECT COMPARED TO A COMMERCIALLY AVAILABLE TCP-CASO4?
F Allen F*, G Blunn, ID McCarthy, M O'Donnell, MM Stevens, AE Goodship
London

17:35 OC28
INTRA-ARTICULAR AMPA/KAINATE GLUTAMATE RECEPTOR ANTAGONISTS ALLEVIATE INFLAMMATION AND PAIN IN RAT ANTIGEN INDUCED ARTHRITIS
CS Bonnet*, AS Williams, DJ Mason
Cardiff

17:45 **Satellite 2**
Supported by Amgen

A Force Against Fracture: New Perspectives in Preventing Bone Loss

West Road Concert Hall

Chair: Juliet Compston (Cambridge, UK)

Graham Russell (Oxford and Sheffield, UK): Bone remodelling and bone strength: what is the correlation?

Jon Tobias (Bristol, UK): Effects of denosumab on the skeleton: a clinical perspective

18:45 End

19:45-22:30 Conference Dinner
Cripps Hall, Queen's College

Wednesday 29th June

09:00 **Oral Communications (BRS)**
(Concurrent with Oral Communications (BORS), below)

Divinity Lecture Theatre

Chairs: David Burr (Indianapolis, USA) & Gavin Clunie (Ipswich, UK)

09:00 OC29
AN INVESTIGATION OF RELATIONSHIPS BETWEEN FRACTURE RISK, TOTAL BODY DXA AND TIBIAL pQCT REVEALS INDEPENDENT INFLUENCES OF AREAL AND CORTICAL BMD: IS THERE A CASE FOR COMBINING DXA AND pQCT IN ASSESSING FRACTURE RISK?
A Sayers*, J Chau, K Tilling, J Tobias
Bristol

09:10 OC30
IRON STATUS, FGF23 AND RICKETS IN THE GAMBIA
V Braithwaite*, LMA Jarjou, GR Goldberg, A Prentice
Cambridge, UK, MRC Keneba, The Gambia

09:20 OC31
TESTING THE MECHANOSTAT THEORY: THE RELATIONSHIP BETWEEN CT BONE AND MUSCLE VARIABLES IN MID-THIGH IN ELDERLY MEN AND WOMEN AND ASSOCIATION WITH INCIDENT LOWER LIMB FRACTURES
F Johannesdottir*, T Aspelund, K Siggeirsdottir, BY Jonsson, B Mogensen, S Sigurdsson, TB Harris, TF Lang, V Gudnason, G Sigurdsson
Reykjavik, Iceland; Kopavogur, Iceland; Malmö, Sweden; Bethesda MD, USA; San-Francisco CA, USA

09:30 OC32
CIRCULATING FIBROBLAST GROWTH FACTOR-23 (FGF-23) FOLLOWING VITAMIN D SUPPLEMENTATION IN VITAMIN D INSUFFICIENCY
R Inaoui*, WD Fraser, I Fogelman,G Hampson
London; Liverpool

09:40 OC33
OBESITY AND FRACTURES IN POSTMENOPAUSAL WOMEN: THE GLOBAL STUDY OF OSTEOPOROSIS IN WOMEN (GLOW)
JE Compston*, et al
Cambridge …(see abstract)

09:50 OC34
RELATIONSHIPS BETWEEN FAT MASS, PLASMA ADIPOKINES AND BONE IN POST-MENOPAUSAL CAUCASIAN WOMEN
KA Ward*, REB Webb, A Prentice, GR Goldberg
Cambridge

10:00 OC35
THE HIGH BONE MASS PHENOTYPE IS CHARACTERISED BY INCREASED SUBCUTANEOUS AND INTRA-MUSCULAR FAT, BUT DECREASED MARROW FAT
CL Gregson*, A Sayers, SA Steel, V Lazar, J Rittweger, JH Tobias
Bristol; Hull; Manchester, UK; Cologne, Germany

10:10 Coffee and Posters

09:00 **Oral Communications (BORS)**
(Concurrent with Oral Communications (BRS), above)

West Road Concert Hall

Chairs: Alister Hart (London, UK) & Iain McNamara (Cambridge, UK)

09:00 OC36
ENHANCED WEAR AND CORROSION IN MODULAR TAPERS IN TOTAL HIP REPLACEMENT - AN IN-VITRO BIOMECHANICAL STUDY
J Meswania*, G Biring, C Wylie, J Hua, S Muirhead-Allwood, G Blunn
Stanmore

09:10 OC37
MEASUREMENT OF SKIN CAPACITANCE: A NOVEL METHOD OF DIAGNOSING AUTONOMIC DYSFUNCTION IN CARPAL TUNNEL SYNDROME
SK Dheerendra*, WS Khan, P Smitham, NJ Goddard
Macclesfield; Stanmore; London

09:20 OC38
T-2 TOXIN DISRUPTS THE EXPRESSION OF CHONDROITIN SULPHATED SULPHATION MOTIFS IN ARTICULAR CARTILAGE FROM AN ANIMAL MODEL OF KASHIN-BECK DISEASE
S Li*, J Chen, B Caterson, CE Hughes
Cardiff, UK; Xi'an, China

09:30 OC39
SPATIAL AND TEMPORAL STUDY OF BONE MARROW LESIONS IN OSTEOARTHRITIS, AND THEIR RELATIONSHIP TO DENUDED ARTICULAR CARTILAGE
SWD McLure*, MA Bowes, CBH Wolstenholme,GR Vincent, S Williams, R Maciewicz, JC Waterton, AJ Holmes, P Conaghan
Leeds; Macclesfield

09:40 OC40
THE OPTIMISATION OF POLYMER TYPE AND CHAIN LENGTH FOR USE AS A BIOLOGICAL COMPOSITE GRAFT IN IMPACTION BONE GRAFTING: A MECHANICAL AND BIO-COMPATIBILITY ANALYSIS
ER Tayton*, S Fahmy, A Aarvold, JO Smith, S Kalra, A Briscoe,M Purcell, KM Shakesheff, S Howdle, DG Dunlop, ROC Oreffo
Southampton; Nottingham

09:50 OC41
COMBINED INFLUENCE OF A CONTINUOUS FLOW BIOREACTOR AND BUFFER SYSTEMS ON CHONDROCYTE PROLIFERATION AND ARTICULAR CARTILAGE TISSUE ACCUMULATION IN LONG TERM CULTURE
AA Khan*, DC Surrao, SD Waldman
Oxford, UK; Queen's University, Canada

10:00 OC42
DOES BONE MARROW ASPIRATE AUGMENT BONE FORMATION WITHIN A HYDROXYAPATITE SCAFFOLD?
MJ Coathup*, W-J Lo, T Edwards, GW Blunn
London; Nottingham

10:10 Coffee and Posters

11:00 BRS Dent Lecture

West Road Concert Hall

Chairs: Jon Tobias (Bristol, UK) & Allen Goodship (London, UK)

Jonathan Reeve (Cambridge, UK)

11:30 Symposium 6
Joint Development and Disease

West Road Concert Hall

Chairs: Jon Tobias (Bristol, UK) & Allen Goodship (London, UK)

11:30 **Andy Pitsillides** (London, UK): Joint development

11:50 **Mary Goldring** (New York, USA): Heterogeneity of chondrocyte responses in cartilage: Lessons from animal models

12:10 **Bruce Caterson** (Cardiff, UK): Musculoskeletal tissue repair involves a recapitulation of cellular & matrix phenotypic changes involved in normal tissue and organ development

12:30 Panel discussion

12:45 Lunch and posters

13:40 BRS AGM
Divinity Lecture Theatre

13:40 BORS AGM
West Road Concert Hall

14:00 **Oral Communications (BORS)**
(Concurrent with Oral Communications (BRS), below)

West Road Concert Hall

Chairs: Mark Birch (Newcastle, UK) & Richie Gill (Oxford, UK)

14:00 OC43
ENHANCEMENT OF PLA FOR USE IN IMPACTION BONE GRAFTING: THE EFFECT OF PRODUCTION VIA SUPERCRITICAL CO2 DISSOLUTION TO INCREASE POROSITY
ER Tayton*, M Purcell, A Aarvold, JO Smith, S Kalra, A Briscoe,S Fahmy, KM Shakesheff, S Howdle, DG Dunlop, ROC Oreffo
Southampton; Nottingham

14:10 OC44
PROTECTION OF ARTICULAR CARTILAGE AGAINST DRYING BY GLUCOSE-SALINE OR SYNOVIAL FLUID
BT McLintock, CE Banfield, AK Amin, AC Hall*
Edinburgh

14:20 OC45
METAL ON METAL TOTAL HIP REPLACEMENT: INFLUENCE OF HEAD SIZE UNDER ADVERSE HIP SIMULATOR CONDITIONS
M AL-Hajjar*, J Fisher, S Williams, JL Tipper, LM Jennings
Leeds

14:30 OC46
INTERVERTEBRAL DISC FISSURES PROVIDE A LOW-PRESSURE, CHEMICALLY CONDUCIVE MICRO-ENVIRONMENT FOR NERVE IN-GROWTH
M Stefanakis*, J Luo1, P Pollintine, T Ranken, J Harris, P Dolan, M A Adams
Bristol; Roehampton

14:40 OC47
THE EFFECT OF HEAT GENERATED BY BONE CEMENT ON THE VIABILITY OF HUMAN MESENCHYMAL STEM CELLS
Y Reissis*, E Garcia, J Hua, G Blunn
London

14:50 OC48
ULTRASTRUCTURAL CHARACTERISATION OF COBALT/CHROMIUM METAL NANOPARTICLES IN MACROPHAGES AND CONSEQUENT CYTOTOXICITY
Z Xia*, D Murray
Swansea; Oxford

14:00 **Oral Communications (BRS)**
(Concurrent with Oral Communications (BORS), above)

Divinity Lecture Theatre

Chairs: Jacqueline Berry (Manchester, UK) & Isabel Orriss (London, UK)

14:00 OC49
DISCORDANCE BETWEEN CORTICAL AND TRABECULAR BONE PHENOTYPE HIGHLIGHTS THE ROLE OF LOCAL VERSUS CIRCULATING IGF-1 IN THE SOCS2 NULL MOUSE
R Dobie*, C Huesa, RJ van't Hof, VE MacRae, SF Ahmed, C Farquharson
Edinburgh; Glasgow

14:10 OC50
SPIROSTOMUM AMBIGUUM: A PROTOZOAN MODEL FOR PRIMORDIAL MUSCULOSKELETAL EXCHANGE?
PE Garner*, V Fallon, JE Aaron
Leeds

14:20 OC51
NOVEL REGULATION STEPS IN PARATHYROID RECEPTOR ACTIVATION
GO Richards*, DJ Roberts, TM Skerry
Sheffield

14:30 OC52
ACTIVE SHAPE MODELLING FOR HIP FRACTURE PREDICTION
SR Goodyear*, RJ Barr, K Yoshida, RM Aspden, IR Reid, DM Reid, JS Gregory
Aberdeen, UK; Auckland, NZ

14:40 OC53
BONE IN VITRO 3D OSTEOBLAST-OSTEOCYTE CO-CULTURE MODEL
M Vazquez*, BAJ Evans, J Ralphs, D Riccardi, DJ Mason
Cardiff

14:50 OC54
NANOTOPOGRAPHY-INDUCED OSTEOGENIC DIFFERENTIATION OF HUMAN EMBRYONIC AND ADULT SKELETAL STEM CELLS
E Kingham*, MP Tsimbouri, N Gadegaard, MJ Dalby, ROC Oreffo
Southampton; Glasgow

15:00 **Oral Posters**

West Road Concert Hall

Chairs: Melanie Coathup (London, UK) & Alison Gartland (Sheffield, UK)

15:00 P001
AUTOLOGOUS OSTEOCHONDRAL TRANSPLANTATION OF THE TALUS - REGIONAL AND LOCAL CONTACT MECHANICS AND A GRAFT HEIGHT ANALYSIS - A BIOMECHANICAL STUDY
AM Fansa, CD Murawski*, CW Imhauser, JT Nguyen, S Schafmeister, JG Kennedy
New York

15:05 P002
HOW MUCH CASUAL SUNSHINE IS ENOUGH FOR ADEQUATE VITAMIN D STATUS
JL Berry*, LE Rhodes, R Kift, AR Webb
Manchester

15:10 P003
CHANGES IN MOUSE GAIT MAY PREDICT OA-LIKE LESION PROGRESSION
RL de Souza*[1,2], B Poulet, AA Pitsillides
London, UK; Mato Grosso, Brazil

15:15 P004
VISUALISATION OF THE APICAL SURFACE OF ACTIVATED OSTEOCLASTS
KA Szewczyk*, K Fuller, RF Moss, TJ Chambers
London

15:20 P005
A TISSUE ENGINEERING APPROACH WITH TANTALUM TRABECULAR METAL TO ENHANCE BONE-IMPLANT INTEGRATION
JO Smith*, BG Sengers, A Aarvold, ER Tayton, DG Dunlop, ROC Oreffo
Southampton

15:25 P006
HAPLOINSUFFICIENCY OF BAG-1 AFFECTS CARTILAGE DEVELOPMENT AND OSTEOGENIC DIFFERENTIATION IN VITRO
RS Tare*, PA Townsend, GK Packham, ROC Oreffo
Southampton

15:30 P007
CHONDROGIDE VERSUS PERIOSTEUM: A HISTOLOGICAL ANALYSIS
HS McCarthy*, S Roberts
Oswestry

15:35 P008
TEMPORAL ANALYSIS OF EMBRYONIC BONE DEVELOPMENT USING AN INNOVATIVE ORGANOTYPIC BONE CULTURE MODEL - APPLICATION OF MICRO-COMPUTED TOMOGRAPHY
JM Kanczler*, EL Smith, CA Roberts, ROC Oreffo
Southampton

15:40 P009
HOW DOES VERTEBROPLASTY AFFECT ADJACENT VERTEBRAE?
J Luo*, DJ Annesley-Williams, MA Adams, P Dolan
Nottingham

15:45 P010
MODULATION OF ENDOGENOUS FPP SYNTHASE CAUSES BISPHOSPHONATE RESISTANCE IN CULTURED CELLS
S Das*, JC Crockett, MJ Rogers
Aberdeen

15:50 P011
VOLUMETRIC WEAR ASSESSMENT OF FAILED METAL-ON-METAL HIP RESURFACING PROSTHESES
JK Lord, DJ Langton, AVF Nargol, TJ Joyce*
Newcastle upon Tyne; Stockton

15:55 P012
THE EFFECT OF HYPERBARIC OXYGEN THERAPY ON OSTEOCLAST FORMATION AND BONE RESORPTION
HW AL-Hadi*,SW Fox,GR Smerdon
Plymouth

16:00 Awards

16 :15 End of meeting

Buses to Cambridge Station

INVITED SPEAKER ABSTRACTS

OSTEOINDUCTION OF BONE GRAFT SUBSTITUTE MATERIALS

G Blunn
John Scales Centre for Biomedical Engineering, Institute of Orthopaedics and Musculoskeletal Science,
Royal National Orthopaedic Hospital, University College London.

Most bone graft substitute materials induce bone formation by osteoconduction. However, in situations such as spinal fusions, bone formation occurs adjacent to the vertebra in regions where bone would not normally form.

Cytokines such as BMPs induce bone formation in non-boney sites. It has also been shown that the structure and topography of biomaterials can influence new bone formation. Evidence indicates that some bone graft substitute materials are osteoinductive and that this osteoinductivity is associated both with the chemistry and with the morphology of the bone graft substitute. For example, incorporating silicon into the crystal lattice of hydroxyapatite increases osteoinductivity as well as osteoconductivity.

The morphology of the bone graft substitute influences bone formation in two ways. Firstly, within the macropores intramembraneous bone formation occurs preferentially on the surface of the implant and bone is deposited in a centrifugal manner filling in the pore. Secondly, increasing the strut porosity within the bone graft substitute increases the osteoinductivity independently of the chemistry. Where both the strut porosity and the chemistry of the bone graft substitute have been optimised, osteoinductivity can be relatively high, accounting for over 30% of available bone ingrowth area within the bone graft substitute. In these bone graft substitutes bone formation occurs within pores of less than 50 microns in size. The osteoinductive nature of some bone graft substitutes is also able to enhance their osteogenic performance, increasing bone formation in bony defects.

CELL AUGMENTATION OF BONE GRAFTS AND BONE GRAFT SUBSTITUTES

R A Brooks
Orthopaedic Research Unit, University Of Cambridge, UK

Bone grafting is commonly used for the repair of bone defects and autologous bone is widely recognised as producing the best clinical outcome which is due in part to its possession of a cellular component. Allogeneic bone and a range of bone graft substitute materials are used in situations where autograft cannot be used or is not available in sufficient quantity however, unlike autogeneic bone these materials have no cellular component and rely on the host bed to provide osteogenic activity. These materials could potentially benefit from the addition of appropriate cells with the aim of increasing the rate of defect repair or improving the quality of the repair tissue and achieving complete bone regeneration. The addition of allogeneic cells is unsuitable for bone repair because of the risk of eliciting an immunological response. This still leaves several potential sources of cells which could be used including those present in the patients' own blood, bone marrow, periosteum and adipose tissue. Cells isolated from umbilical cord could also be harvested and stored for future use as a supply of autologous cells. Platelets and bone marrow have both been used clinically but there is as yet a paucity of data from clinical trials. The cells in bone marrow proposed as providing osteogenic activity are the mesenchymal stem cells and their differentiated progeny and there has been a lot of research aimed at isolating, characterising and expanding the numbers of these cells in culture for therapeutic use however so far their use in bone repair and regeneration has been limited to preclinical research and experimental medicine. Recently research demonstrating the ability to induce pluripotency in adult differentiated cells has led to optimism that induced pluripotent stem cells will prove valuable for bone tissue regeneration as they offer the potential for an unlimited supply of cells. This talk will outline the evidence so far for the real and potential benefits of cell augmentation in bone repair and propose some areas for further investigation.

A STRUCTURAL ENGINEER'S VIEW OF BONE MECHANICS

C Burgoyne
Head of Structures Group, Cambridge University Engineering Department

Structural engineers are concerned with the physical properties of the materials they use, principally elasticity, strength and toughness. Their structures obey the laws of equilibrium and compatibility, which are essentially physics; chemistry is rarely an issue and biology never. Bone mechanicians start from the opposite end; they start with biology, know a lot of chemistry, and a little physics. There is little meeting of minds between the two groups and almost no common language. Biological structures do not look like engineered structures, yet both are governed by the same physical laws.

This presentation will consider why engineered structures are the shape they are; we will consider the three "S"s – Strength, Stiffness and Stability. We will see how engineers take all three into consideration when designing structures, and how failure to deal with interaction between them has led to some major structural failures.

We will then consider how the same principles apply to bone, but we will now add other considerations. It will be taken as axiomatic that evolution has led to biological structures that are (in some sense) optimal. It is also recognised that we don't have enough genes even to provide a full blueprint for a fully-grown adult, let alone detailed strategies for what happens if a particular part of the skeleton is damaged. What must be encoded are *processes* that produce the self-healing optimal structures that we are.

Examples will be given of how structural mechanics informs us about the behaviour of bone. How can the complex structure of some bones be explained by considerations of the forces that they must resist? What does a failure on the Hubble Space Telescope have in common with some dinosaur bones? Why is stability rarely a consideration in bone mechanics, but when might it be important?

It is clear that the laws of physics apply to biological structures in the same way that they apply to a bridge. They control not only the overall shape but the internal structure of bones and must be considered alongside the biology and chemistry more familiar to bone biologists.

SKELETAL MICRODAMAGE: GOOD, BAD, OR PHYSIOLOGIC?

D B Burr
Indiana University School of Medicine, Indianapolis, IN, USA

Skeletal microdamage has been implicated as a predisposing factor to fracture, but this is an overly simplistic view. While it is true that damage accumulation in bone reduces residual strength, stiffness and energy to fracture, the creation of new cracks, and their growth, both dissipate energy and delay fracture. While microdamage has contrasting mechanical consequences, it has clear physiologic significance in signaling through osteocytes for targeted bone remodeling. Several groups have shown that osteocyte apoptosis is key to the initiation of remodeling, and preventing apoptosis will prevent the repair of microdamage. Therefore, it may be more productive to begin to consider skeletal microdamage not only in mechanical terms, but in physiologic terms. When remodeling is artificially suppressed, as it is when anti-catabolic agents are used to control bone loss in post-menopausal osteoporosis, microdamage naturally accumulates, and accumulates in an exponential fashion with greater reductions in bone turnover. This accumulation results from a combination of increased probability for crack initiation, as well as the reduced opportunity for crack repair. One reason for the increased likelihood of crack initiation is that reduced bone remodeling allows post-translational modifications to collagen that reduce its ductility under load. These changes do not alter pre-yield deformation, but reduce the post-yield work to fracture. The initiation of new cracks may delay eventual fracture under these circumstances. Therefore, although crack accumulation associated with treatments for

osteoporosis has generally been viewed negatively, we must consider the possibility that this is an adaptive mechanism which delays fracture in the face of increasingly brittle tissue properties.

MUSCULOSKELETAL TISSUE REPAIR INVOLVES A RECAPITULATION OF CELLULAR & MATRIX PHENOTYPIC CHANGES INVOLVED IN NORMAL TISSUE AND ORGAN DEVELOPMENT

B Caterson
School of Biosciences, Cardiff University

There is an increasing literature that indicates that metabolic processes used for the repair of injured or diseased adult tissues often involve cellular, molecular & matrix deposition events that recapitulate those that were initially used in the development of immature organs & tissues; this is particularly the case for musculoskeletal tissues. For the past several years our lab has performed many studies investigating the biological function and role(s) that the very specific and dynamic expression of chondroitin sulphate/dermatan sulphate (CS/DS) sulphation motifs on glycosaminoglycans (GAGs) associated with cell surface & matrix proteoglycans play during different developmental stages of musculoskeletal tissues & organs. These studies have used a panel of monoclonal antibodies (mAbs) that specifically recognise 'native' CS/DS sulphation motifs in CS/DS GAGs. Using these mAbs we have noted that there is a dynamic spatio-temporal expression of these CS/DS motifs occur on & around stem/progenitor cell niches during development which leads us to hypothesise that they are involved in the formation of morphogen/growth factor gradients used in tissue development. Interestingly, we also find that these CS/DS motifs are expressed on & around 'cell clusters' that are often observed in injured or diseased musculoskeletal tissue; e.g. in osteoarthritic cartilage & in degenerate intervertebral disc tissues. We hypothesise that the appearance of these 'cell clusters' is an indicator of attempts from endogenous stem/progenitor cells to repair the injured or diseased tissue. However, this tissue repair often does not succeed because of the inability of the already compromised 'adult tissue' to cope with the natural mechanical forces experienced in adult versus adolescent musculoskeletal tissue functions. Currently, our lab is investigating whether or not the specific isolation of these stem/progenitor 'cell clusters' from diseased or injured tissues (and their *ex vivo* expansion) may afford us a useful source of autologous stem/progenitor cells for use in tissue engineering and regeneration procedures for injured or diseased musculoskeletal tissues.

ENERGY METABOLISM AND BONE

C Chenu
Royal Veterinary College, London

The intimate association between fat and bone has long been demonstrated based on the fact that cells which are important in obesity (adipocytes) and osteoporosis (osteoblasts) share a common cell progenitor and interact with each other via endocrine pathways. Over the last decade our current view of the relationship between the skeleton and energy metabolism has changed due, first to the discovery of a central control of bone remodeling, and more recently, to the demonstration that bone itself may be involved in the control of energy metabolism by producing osteocalcin which regulates insulin production and sensitivity. This field of research is rapidly expanding towards different directions for a better understanding of the interactions between the skeleton and energy metabolisms. Our current investigations have revealed that adenosine 5'-monophosphate-activated protein kinase (AMPK), a key sensing mechanism in the regulation of cellular energy homeostasis and an essential mediator of the central and peripheral effects of many appetite-regulating hormones, plays an important role in bone physiology. *In vitro* studies demonstrate that AMPK activators stimulate bone formation while inhibiting osteoclast formation and bone resorption. Genetic studies have also shown that AMPK is important for the maintenance of bone mass *in vivo*. AMPK is a key molecule in controlling metabolic diseases such as type 2-diabetes and obesity and is activated by antidiabetic drugs such as metformin and thiazolidinediones (TZDs) which also regulate bone cell functions and skeletal mass. Furthermore, AMPK activity can be regulated in bone cells by these same hormones that regulate food intake and energy expenditure through AMPK activation in the brain and peripheral tissues. Due to its regulation of the coordination between anabolic and catabolic metabolic pathways, AMPK signaling represents an attractive therapeutic target for interventions in many conditions of disordered energy balance, including osteoporosis.

ATYPICAL FEMORAL FRACTURES

J Compston
Professor of Bone Medicine, Cambridge University Hospitals NHS Foundation Trust, Cambridge UK

Over the past five to six years there have been a number of reports of so-called atypical femoral fractures occurring in patients treated with bisphosphonates. These fractures have highly characteristic clinical and radiological features: they occur in the subtrochanteric or diaphyeal region of the femur and are transverse or simple oblique, they are associated with lateral cortical thickening and "beaking" of the medial cortex; they may be bilateral; they are often preceded by prodromal pain or weeks or even months; and in many cases healing is delayed.

There is still some uncertainty as to whether atypical femoral fractures represent part of the spectrum of osteoporotic fractures or whether they are specifically related to bisphosphonate therapy. The recently reported correlation between duration of bisphosphonate therapy and risk of atypical fractures supports a causal association, as does the observation that whilst the age-adjusted incidence of hip fracture is falling, atypical fractures are becoming more common. The mechanism most commonly proposed is long-term suppression of bone turnover, although to date these fractures have not been associated with anti-resorptive drugs other than bisphosphonates. Further studies are required to define the pathophysiology of these fractures and their relationship, if any, to bisphosphonate therapy. Even if a causal relationship does exist, the risk benefit ratio of bisphosphonate therapy remains overwhelmingly positive in individuals at high risk of fracture.

THE 4MS: MATERIALS, MINERALISATION, MICRODAMAGE AND MECHANICS: A BIOLOGIST'S VIEW

J Currey, York

What are bones designed to do? Our bodily make-up is determined mostly by our hunter-gatherer ancestors. Failure is different in different circumstances. For a toddler 'failing' means breaking a bone, for her father running after wounded prey, bone stiffness, resulting in efficient locomotion, is all important. However bone cannot be both stiff and tough. One could make a tough material into a stiff bone by making the bone fat, but this would be no good for the father; the heavy bone would slow him down. The ear bones have different requirements from the long bones, and being more highly mineralised they are much stiffer, but also more brittle. All bone design has to produce systems of minimum weight.

It is a surprising fact that the safety factor found in bones of many species of bird and mammal are very similar. That is, the strength of the bone is only about 3 times as great as the stresses imposed during extreme activities. However, natural selection is only concerned with maximising the number of children and grandchildren a person will have, and safety has often to be sacrificed for efficiency.

The design of bones shows that weight is bad. Bones are hollow (though filled with the least dense packing material available, fat) because hollowness increases their stiffness-to-weight ratio. Similarly, flat bones such as the scapula have a sandwich construction with two sheets of compact bone separated by cancellous bone, although this is filled with marrow fat. Sandwiches have a small stiffness-to-weight advantage over solid bone. Manatees have an

opposite weight problem, finding difficulty in getting down a couple of metres below the surface of the sea to graze, and have solid flat bones, and solid ribs.

Ageing people are evolutionarily dead. In general the diseases of people over the age of about 45 are of little importance in the great scheme of things, because their reproductive potential is very small. After 45 you are freewheeling downhill.

BONE REMODELING: HOW ARE BONE RESORPTION AND FORMATION INTEGRATED IN A JOINT BONE RENEWAL PROGRAM?

J-M Delaisse
Professor of Clinical Cell Biology, University of Southern Denmark (Odense), Denmark

A remarkable property of bone remodeling is that osteoblasts (OB) restitute exactly the bone matrix removed by osteoclasts (OC), in so-called bone remodeling units. This indicates a strict coordination of OC and OB activities. What is the mechanism? This question remains unanswered despite extensive research on OCs and OBs. Our analysis of the supracellular organization of bone remodeling units in biopsies of human trabecular bone draws the attention on two likely players in OC-OB coordination, which are most often overlooked.

First, OCs and bone forming OBs appear always separated by mononucleated cells lining eroded surfaces, which are called reversal cells. This critical position makes them privileged candidates for coupling bone resorption to formation, and suggests their involvement in recruitment and differentiation of OBs, or in preparation of the eroded bone surface for matrix deposition. They cover more than 80% of the eroded surface of control bones, but their actual nature and significance for bone remodeling are still unknown. We now found that they represent a heterogeneous population of OB lineage cells and that flattening of these cells correlates with arrest of the reversal phase and deficient bone formation in postmenopausal osteoporosis.

Second, OCs and most bone forming OBs from control biopsies are covered by a canopy of flat OB-like cells, which separates OCs and bone forming OBs from the bone marrow cavity. This canopy generates a so-called bone remodeling compartment (BRC) representing the anatomical structure where the many diverse signals regulating OCs and OBs are integrated. These canopies are closely associated with capillaries. This association allows fast access of systemic regulators, possible guidance of OC and OB progenitors to critical points of the bone surface, and may provide an environment promoting maturation of these progenitors. Disruption of canopies above reversal surfaces in postmenopausal osteoporosis, Cushing, and multiple myeloma, coincides with absence of transition of the reversal phase to bone formation.

Our observations support an effective role of reversal cells and BRC canopies in coupling OC bone resorption to OB bone formation, and stress the need for investigating the microenvironment of bone remodeling units.

THE 4MS: MATERIALS, MINERALISATION, MICRODAMAGE AND MECHANICS: THE BONE MATERIAL

M Kerschnitzki[1,4],C Lange[1,4], W Wagermaier[1], P Roschger[2], K Klaushofer[2], G N Duda[3,4], P Fratzl*[1,4],
[1]Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam, Germany; [2]Ludwig Boltzmann Institute of Osteology at Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling,1st Medical Department, Hanusch Hospital, Vienna , Austria; [3]Julius Wolff Institute and Center for Musculoskeletal Surgery, Charité University Hospital, Berlin; [4]Berlin-Brandenburg School of Regenerative Therapies, Berlin, Germany

Bone is a hierarchically structured material and its fracture properties depend on many length scales. In particular, the mineral content and its distribution, as well as the size and arrangement of mineral particles play a crucial role for the bone material properties. Due to growth and remodelling, these mineral characteristics vary locally and with time. In addition, fracture risk does not only increase with reduced bone mass, which is considered a hall mark of osteoporosis, but also depends on the material properties, that is, on the status of the mineral and the organic matrix. Recent studies of developing and healing bone tissue reveal an even greater heterogeneity than previously expected. In particular, collagen and mineral alignment within bone tissue mirrors the structure of the osteocyte network. Highly ordered lamellar bone tissue corresponds to planar arrangements of osteocytes, which seem to organise on top of earlier, poorly ordered bone tissue. Size, shape and arrangement of mineral particles vary greatly between these tissue types and contribute to the complex mechanical behaviour of the bone material.

HETEROGENEITY OF CHONDROCYTE RESPONSES IN CARTILAGE: LESSONS FROM ANIMAL MODELS

M B Goldring
Senior Scientist, The Hospital for Special Surgery
Professor of Cell & Developmental Biology, Weill Cornell Medical College, USA

Proteins produced in response to excessive mechanical loading and inflammation in joints not only stimulate the production of enzymes that break down the cartilage but also impair the ability of the chondrocyte, the unique cell type in adult cartilage, to repair the damage. We have used several strategies for identifying and characterizing mediators involved in the pathogenesis of osteoarthritis (OA), including culture models of primary human and mouse chondrocytes and cell lines, mouse models, and human cartilage samples. Human cartilage is a complex tissue of matrix proteins that varies from superficial to deep layers and from loaded to unloaded zones. During OA development the normally quiescent chondrocytes with low matrix turnover undergo phenotypic modulation resulting in matrix destruction and abnormal repair. We have identified new genes, not known previously to act in cartilage, including growth arrest and DNA damage (GADD)-45□, induced in chondrocytes by bone morphogenetic protein (BMP)-2, and the ETS transcription factor, ESE1/ELF3, induced by inflammatory cytokines. Both GADD45□ and ESE1/ELF3 are induced by NF-□B and, in turn, upregulate matrix metalloproteinase (MMP)-13 and suppress type II collagen (COL2A1) gene expression. We discovered a novel role for GADD45□, an anti-apoptotic factor during genotoxic stress and cell cycle arrest, as a mediator of MMP-13 and Col10a1 gene expression during embryonic growth plate development. Since GADD45□ is present in quiescent chondrocytes in normal cartilage and in early OA cartilage at sites peripheral to the lesion in chondrocyte clusters, in deep zone chondrocytes, and in osteophytes, it may promote chondrocyte survival, while promoting cartilage calcification and osteophyte formation. Current studies involve both in vitro analysis of signaling and transcriptional mechanisms that regulate the expression and activities of GADD45□ and ESE-1 and in vivo analysis of the consequences of knockout and transgenic overexpression of these genes in mouse models, using surgical OA (good matrix with abnormal loading) and genetic models with OA-like pathology (bad matrix with normal loading) during aging. In further studies, we are examining the epigenetic regulation of MMP-13 and using proteomics and genomics approaches to map the signaling networks and microRNA targets that impact on gene expression programs during the onset and progression of OA. These studies may to lead to identification of critical targets for therapy to block cartilage damage and promote effective cartilage repair.

THE BONE AND JOINT DECADE: CLOSED CHAPTER OR OPEN BOOK?

OSTEOPOROSIS

J A Kanis
WHO Collaborating Centre, University of Sheffield, UK

The past decade has seen substantial advances in our knowledge of osteoporosis. These include an accurate description of the disease and

quantification of the morbidity, mortality and financial cost to society that result from fragility fractures. In Europe, osteoporotic fractures account for more by disability adjusted life years (DALYs) lost than any cancer, with the exception of lung cancer. The DALYs lost in Europe from osteoporosis (2.0 million) were less than for osteoarthritis (3.1 million), but greater than for rheumatoid arthritis, ranking the disorder high in the league table of non-communicable diseases.

Against this background there has been an increasing therapeutic armamentarium available to decrease the risk of fracture. Major pharmacological interventions are the bisphosphonates, strontium ranelate, raloxifene, denosumab and parathyroid hormone peptides, several of which have been developed directly from the application of acquired knowledge of bone biology. Well designed placebo controlled clinical trials have shown that they reduce the risk of osteoporotic fractures. The reduction in vertebral fracture rate has generally been between 50 and 70% whereas the magnitude of reduction in non-vertebral fracture, where demonstrated, has generally been smaller and in the order of 15-25%.

A major challenge has been how to apply these treatments. Measurements of bone mineral density (BMD) are used for diagnosis and for fracture risk prediction. Facilities for BMD testing are patchy and many countries, including the UK have inadequate resources to service the societal needs. In addition, BMD has poor sensitivity for the prediction of fracture so that the majority of fractures occur in individuals with T-scores >-2.5 SD. The development of FRAX has improved the sensitivity of fracture risk prediction. FRAX uses easily obtainable clinical risk factors to estimate 10-year fracture probability, with or without femoral neck bone mineral density. It has been constructed using primary data from population-based cohorts around the world. The gradients of fracture risk have been validated externally in independent cohorts. FRAX tools are currently available for 31 countries and been incorporated into many practice guidelines.

Despite these advances, there are a number of challenges to be faced. Of paramount importance is that few patients with a prior fracture and even less with osteoporosis alone actually receive treatment. Thus the disease is under-recognised by the medical community. The development of fracture liaison services could potentially alleviate this problem. The fracture that occasions the greatest morbidity and mortality is hip fracture, so that treatments that do not reduce hip fracture risk have a limited impact on the burden of disease. A further challenge is that compliance with treatment is poor. In many studies, less than 50% of patients are still taking medication one year after its prescription. This is a problem world wide which not only reduces effectiveness of treatment, but also increases cost. There is some evidence that intermittent treatment (e.g. once weekly, monthly, yearly etc) improves compliance, but the major determinants of compliance are unknown.

The frequency of osteoporotic fracture is rising in many countries. Reasons for this relate in part to the increased longevity of the population, which is occurring both in the developed and underdeveloped world. In Europe the total population will not increase markedly over the next 25 years, but the proportion accounted for by the elderly will increase by 33%. In Asia, the total population as well as life expectancy of the elderly will increase two-fold over the next 25 years, so that osteoporotic fractures will assume even greater significance for health care planning. Over and above the increasing population at risk, there is an increase in age and sex specific incidence in many communities. Thus, the numbers of hip fractures will more than double assuming no change in age-specific risk, but will more than quadruple with very conservative estimates of the secular trend. The development of effective strategies for the primary and secondary prevention of fragility fractures are real challenges for the next decade.

PREVENTING THE SECOND HIP FRACTURE, WHAT DRUGS TO USE AND WHEN

RW Keen
Royal National Orthopaedic Hospital, Stanmore. UK

Patients who sustain a hip fracture are at increased risk for further fracture. Up to 1 in 20 hip fracture patients will sustain a further hip fracture within one year of their initial injury. It is therefore important that this high risk group of patients are managed effectively.

The following drugs have Grade A evidence to support a reduction in hip fracture risk: Alendronate, Denosumab, Hormone replacement therapy, Risedronate, Strontium Ranelate, and Zoledronate. Only, however, with Zoledronate has the drug been studied specifically in a group of men and women sustaining an index hip fracture. In a randomised, double-blind, placebo-controlled trial, 2127 patients were assigned to receive yearly Zoledronate 5mg or placebo. The infusions were first administered within 90 days after surgical repair of a hip fracture. The rates of any new clinical fracture and any new non-vertebral fracture were significantly reduced in the Zoledronate group compared to the placebo group. The results for new hip fracture were, however, not statistically significant. Post-hoc analysis has demonstrated that the benefits on fracture and mortality were only seen in patients receiving treatment at least 2 weeks after their initial fracture.

This presentation will review the data supporting the use of the drugs in patients who have sustained a hip fracture. Discussion will be given to the merits of anti-resorptive treatment versus anabolic. The presentation will also examine the side-effect profiles of the various treatments, and how these may influence the choice of drug in clinical practice.

Eriksen EF, Lyles KW, Colón-Emeric CS, et al. Antifracture efficacy and reduction of mortality in relation to timing of the first dose of zoledronic acid after hip fracture. J Bone Miner Res. 2009;24:1308-13.

Lloyd BD, Williamson DA, Singh NA, et al. Recurrent and injurious falls in the year following hip fracture: a prospective study of incidence and risk factors from the Sarcopenia and Hip Fracture study. J Gerontol A Biol Sci Med Sci. 2009;64:599-609.

Lyles KW, Colón-Emeric CS, Magaziner JS, et al. Zoledronic acid and clinical fractures and mortality after hip fracture. N Engl J Med. 2007;357:1799-809.

JOINT DEVELOPMENT

A A Pitsillides
Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK

Development of synovial joints is usually divided into two phases; first, the formation of cartilaginous precursors of the opposing bones and the intervening interzones in which the joints will develop, and second the formation of the articular cartilage, synovium and other related joint structures along with formation of the joint cavity. Here, we will review current mechanisms by which these events are thought to be achieved. We will discuss whether skeletal elements are discrete entities from the outset or an uninterrupted cartilage matrix is divided later, whether articular chondrocytes are derived from the distinct cohort of cells and finally also examine data focussing on how these events might inform our understanding of the joint cavity-forming process (cavitation). Particular attention will be focused on the role played by mechanical stimuli. Although it is frequently asserted that cavitation involves localised cell death; critical evaluation will question the validity of this assertion. Our work will also highlight novel roles for constitutively active extracellular-regulated kinase, $p38^{MAPK}$ and the inducible isoform of cyclo-oxygenase, COX-2 in local signalling required for the elaboration of the hyaluronan-rich ECM essential for efficient cavity formation. Finally, clues from studying glycosaminoglycan (GAG) synthesis during the mechano-dependent joint formation process will be pursued to examine whether novel modes for controlling cartilage GAG content may be revealed.

PHARMACOLOGICAL TARGETS IN AGEING BONE

G Russell
The Botnar Research Centre, Oxford University Institute of Musculoskeletal Sciences, Nuffield Orthopaedic Centre, Headington, Oxford, UK

There have been remarkable recent advances in knowledge about skeletal biology and the regulatory systems controlling bone formation and resorption, in development and with ageing. These are largely the result of discoveries in genetics and cell biology. These advances are not only leading to a better understanding of the pathophysiological basis of bone diseases, but also to identifying new targets that allow novel approaches to therapy.

Among the important recently discovered pathways that are involved in osteoblast regulation and osteoblast/osteoclast interactions and are amenable to pharmacological intervention are the wnt/LRP5 pathway, and particularly the RANK Ligand/RANK/OPG system. Other local regulators include numerous cytokines, transcription factors and the ephrin system. At a systemic level there is evidence that products of bone cells may have distant endocrine effects. Indeed osteocytes have their own repetoire of regulatory molecules, including FGF23, which is involved in phosphate metabolism, and sclerostin, which is a powerful negative regulator of bone formation. Furthermore there is some evidence that osteocalcin, secreted by osteoblasts, may act as a systemic metabolic regulator by controlling insulin secretion, and insulin sensitivity. There are also potential regulatory links between the hypothalamus, the gut, and adipose tissue involving leptins, serotonin, and adipokines, the clinical importance of which are still unclear.

The translation of thèse findings into clinical use is progressing rapidly. Denosumab, an anti-RANKL antibody, has already been approved for treatment of osteoporosis and bone metastases, and antibodies directed against sclerostin are showing promise as potential anabolics. A similar approach can be used to block *dkk* to activate *wnt* signalling, thereby stimulating bone formation, as being tested in myeloma. Inhibition of the bone specific protease cathepsin K is another approach and drugs such as odanacatib are in clinical development. Drug development is a slow and costly procèss, so for the near future already established drugs, especially the bisphosphonates, are likely to continue to dominate the market. The molecular actions of nitrogen-BPs are now well understood as potent inhibitors of FPPS (farnesyl pyrophosphate synthase), which has highlighted the importance of the mevalonate pathway in regulating osteoclast function, New approaches to target this pathway are under study.

NEW MATERIALS BASED STRATEGIES FOR BONE REPAIR

M M Stevens
Departments of Materials and Bioengineering, Imperial College London, UK

This talk will provide an overview of our recent developments in bio-inspired materials and tissue regeneration of bone. Engineering of large-volumes of bone in vivo will be presented using relatively simple yet effective biomaterials approaches based on hydrogels or strontium containing bioceramics. Strontium ranelate has found great success as an oral anti-osteoporosis drug with effects on both osteoblasts and osteoclasts. The development of modified poly-γ-glutamic acid based materials that can be processed to generate materials with a range of mechanical properties appropriate for ligament and other tissue repair will also be discussed. Additionally a thorough materials analysis of tissue engineered bone will be presented. Indeed many different cells are used in bone regeneration applications but it is not always clear if they produce a material that mimics the structural and compositional complexity of native bone. By applying multivariate analysis techniques to micro-Raman spectra of mineralized nodules formed in vitro, we have revealed cell-source-dependent differences in interactions between multiple bone-like mineral environments. These recent findings will be discussed here. Understanding the biological mechanisms of bone formation in vitro that contribute to cell-source-specific materials differences may facilitate the development of clinically successful engineered bone.

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OBESITY AND BONE IN CHILDHOOD

JH Tobias
Musculoskeletal Research Unit, University of Bristol

Relationships between obesity and bone in childhood are complex and controversial. In the largest cross sectional study of the relationship between fat and bone mass in childhood, based on total body DXA scans in around 3000 subjects mean 9.9 years of age from the Avon Longitudinal Study of Parents and Children (ALSPAC), we observed a positive association between fat and bone mass in analyses adjusted for height and weight. This appeared to reflect an influence on overall bone size, and though stronger for lower limb bone area, an association was also seen between fat mass and upper limb bone area, suggesting the role of some kind of systemic influence over and above a simple effect of greater mechanical loading due to excess weight. Subsequent analyses where we applied an instrumental variable analysis approach to overcome potential confounding revealed similar findings.

Further studies based on more detailed analysis of bone structure have partly supported these results, with a positive relationship between fat mass and tibial periosteal circumference seen in ALSPAC as assessed by pQCT at 15 years. On the other hand, in the GOOD cohort of young adult men, whereas similar results were obtained at the tibia, this was not seen at the radius, suggesting that mechanical strain in fact contributes a major part to the positive influence of fat mass on bone accrual. Consistent with this view, in further analyses where we examined the role of adiponectin as a possible systemic mediator, we observed that whereas adiponectin is a negative regulator of bone growth in childhood as assessed by pQCT, this action is largely independent of fat mass.

The relationship between obesity and bone may also be affected by site of fat deposition. Although visceral fat mass contributes a relatively small proportion to overall fat mass, in studies where this has been measured directly using methods such as MRI and CT, an inverse association is generally observed with indices of bone development, in contrast to measures of subcutaneous fat. Metabolic disturbances such as insulin resistance have been suggested to be inversely related to bone mass in adolescents, which may be explained by their association with visceral adipose tissue. Preliminary findings in ALSPAC suggest that a similar negative pathway may exist between insulin resistance, bone development and intra-muscular fat mass, of which the latter was based on muscle density as measured by pQCT. Although the mechanisms by which visceral and intramuscular fat exert an apparent inhibitory influence on bone development are currently unclear, inflammatory cytokines contributing to the increased risk of cardiovascular disease in this context might also be expected to affect bone development.

ADIPOSE TISSUE EXPANDABILITY, LIPOTOXICITY AND THE METABOLIC SYNDROME

A Vidal-Puig
Department of Clinical Biochemistry, University of Cambridge

The link between obesity and type 2 diabetes is clear on an epidemiological level, however the mechanism linking these two

common disorders is not well defined. One hypothesis linking obesity to type 2 diabetes is the adipose tissue expandability hypothesis. The adipose tissue expandability hypothesis states that a failure in the capacity for adipose tissue expansion, rather than obesity per se is the key factor linking positive energy balance and type 2 diabetes. All individuals possess a maximum capacity for adipose expansion which is determined by both genetic and environmental factors. Once the adipose tissue expansion limit is reached, adipose tissue ceases to store energy efficiently and lipids begin to accumulate in other tissues. Ectopic lipid accumulation in non-adipocyte cells causes lipotoxic insults including insulin resistance, apoptosis and inflammation. This article discusses the links between adipokines, inflammation, adipose tissue expandability and lipotoxicity. Finally, we will discuss how considering the concept of allostasis may enable a better understanding of how diabetes develops and allow the rational design of new anti diabetic treatments.

ORAL COMMUNICATIONS

OC1

OSTEOCYTES PROMOTE OSTEOBLAST PROLIFERATION DIRECTLY AND EXERT A DISTINCT EFFECT WITH MECHANCIAL STRAIN

RFL Suswillo[1], SCF Rawlinson*[2], GP Dowthwaite[1], LE Lanyon[1] and AA Pitsillides[1]

[1]Dept Veterinary Basic Sciences, The Royal Veterinary College, UK; [2]Dept of Oral Growth and Development, Queen Mary University of London, Barts & The London School of Medicine and Dentistry, UK

In contrast to bones of the skull vault, limb bones lose mass and mechanical integrity in low mechanical load environments. The reason for this discrepancy is unknown, but regional differences in the osteocyte (OC) response to mechanical stimuli might be the underlying cause. OCs make no direct contribution to the structural (re)modelling on bone surfaces and it has been hypothesised that their pro-osteogenic influence involves gap junction (GJ) mediated regulation of osteoblast (OB) behaviour.

To examine whether osteocytes influence OB behaviour directly, and whether this is GJ-mediated or modified by mechanical strain (600 cycles, 1 Hz, peak longitudinal strain, 3000microstrain) an in vitro co-culture model was developed using OBs and immunomagnetically isolated primary OCs from tibiotarsi (LOCs and LOBs) and calvariae (COCs and COBs) of 18-day old chick embryos (OCs to OBs, 4:1 ratio).

Accordingly, we first established that OCs are post-mitotic with a characteristic phenotype, that LOBs and COBs show comparable rates of proliferation, and that basal and strain-related increases in proliferation in co-cultures is attributable to OB proliferation alone. We then showed that COB proliferation rates were unaffected by homotypic co-cultures with COCs. In contrast, LOCs induced significant increases in basal proliferation of LOBs. This enhancement of LOB proliferation was independent of GJ number or activity (scanning EM revealed similar cell:cell contacts in both co-cultures and the pro-proliferative influence of LOCs was unaffected by 20microM 18beta-glycyrrhetinic acid, a GJ inhibitor). Interestingly, LOCs also promoted COB proliferation in heterotypic cultures - indicating a fundamental difference in transcellular messaging communicated to OBs by LOCs and COCs. We also found that mechanical strain-related increases in LOB proliferation were, however, restricted by co-culture with LOCs and that this was in part dependent on functional of GJs.

These data demonstrate: i) that OCs derived from limb bones, but not skull bones exert pro-proliferative effects upon OBs, ii) that LOCs also act to restrict strain-related increases in OB proliferation, and iii) that only the later requires communication via gap junctions.

Identification of distinct responses to OC signalling, under basal and mechanically-challenged conditions, provides new insights into their function in bone.

OC2

ELEVATED SCLEROSTIN LEVELS IN INDIVIDUALS WITH HIGH BONE MASS

CL Gregson*[1], KES Poole[2], SA Steel[3], J Ayuk[4], EV McCloskey[5], WD Fraser[6], JH Tobias[1]

[1]Musculoskeletal Research Unit, School of Clinical Sciences, Bristol University, Bristol, UK;[2]Addenbrookes Hospital, Cambridge, UK;[3]Hull Royal Infirmary, Hull, UK;[4]Queen Elizabeth Hospital, Birmingham, UK;[5]Metabolic Bone Centre, Sheffield University, Sheffield, UK;[6]Department of Clinical Biochemistry and Metabolic Medicine, Liverpool University, Liverpool, UK

Sclerosteosis is a rare autosomal recessive disorder associated with High Bone Mass(HBM), caused by a loss-of-function mutation in the SOST gene encoding sclerostin, an osteocyte-derived inhibitor of bone formation. We recently recruited a large cohort of HBM individuals from systematically searching DXA databases around the UK; although to date sequencing has not identified any SOST gene mutations, we hypothesized that disordered sclerostin production might contribute to raised BMD in this context.

406 adult HBM index cases were identified by screening DXA databases from 4 UK centres (n=219,088). HBM was defined as a) L1 Z-score of 3.2 or more and total hip Z-score of 1.2 or more, or b) total hip Z-score 3.2 or more. Cases with significant osteoarthritis and/or other causes of raised BMD were excluded. Relatives and spouses were recruited, in whom HBM affection status was defined as L1 Z plus total hip Z-scores of 3.2 or more. Controls comprised unaffected relatives and spouses. Sclerostin was measured in a randomly selected subgroup of 40 men and 40 women, after age stratification, representing 51 HBM cases (37 index, 14 affected relatives) & 29 controls (22 unaffected relatives, 7 spouses). None had parathyroid disease. Analyses used multiple linear regression in Stata 11.

As expected, sclerostin increased with age in HBM cases and controls. Sclerostin was higher in HBM cases than controls, mean (SD) 109.4 (47.2) vs. 81.2 (38.9)picomoles/litre, p=0.005, which persisted after adjustment for age, sex, weight & height, mean difference 29.2 (7.9, 50.6)picomoles/litre, p=0.008. We hypothesized that elevated sclerostin levels in HBM cases reflects a regulatory response as part of a feedback loop rather than a primary disturbance. Hence, in our HBM cases, we analysed relationships between sclerostin level and total body BMD (measured by DXA) and serum osteocalcin. As predicted, sclerostin levels were positively related to both total body BMD Z-score [16.8 (4.8,28.8), p=0.007] and to osteocalcin [18.6 (3.6,33.6), p=0.016] (results show beta coefficient with 95% confidence interval adjusted for age, sex, weight & height).

We conclude that sclerostin levels are elevated in HBM cases, possibly as a compensatory response to reduced strain associated with having increased bone mass.

OC3

MARKED REDUCTION IN THE EXPRESSION OF SCLEROSTIN IN OSTEOCYTES CLOSE TO THE SITE OF FEMORAL NECK FRACTURES

J. Power[1], M. Parker[3] S. Papapoulos[4], R. van Bezooijen[4] J. Reeve[1], N. Loveridge*[1,2]

[1]Medicine and [2]Orthopaedic Research Unit, University of Cambridge, Cambridge, [3]Surgery, Peterborough City Hospital, Peterborough, United Kingdom, [4]Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, Netherlands

One potential therapy to enhance fracture healing is anti-sclerostin antibodies but it is unclear as to whether sclerostin expression is normally reduced at fracture sites. Although sclerostin mRNA is reduced in tissue taken from fracture cases (PLoS ONE 6: e16947; 2011) its location relative to the fracture site is unclear.

We used biopsies from cases of femoral neck fracture to analyse the spatial expression of sclerostin in osteocytes relative to the fracture surface. In each case, the date of fracture and operation was recorded. The distal stump of the femoral neck (removed to allow insertion of the prosthesis) was rapidly frozen for study; immunolocalisation of sclerostin expression used previously described methods (FASEB J 9:1842-4, 2005).

In all biopsies (n=7), sclerostin expression was markedly reduced close to the fracture surface. To determine if this was related to possible damage during biopsy preparation, cryostat sections were cut at right angles to the fracture plane in a way that contained both a fracture surface and sawn surface (created during arthroplasty) so that each patient acted as his/her own control.

In 2 biopsies, the proportion of osteocytes positive for sclerostin (scl+ve) was measured in 0.2mm steps from fracture and sawn surfaces. Sclerostin expression was reduced close to the fracture surface. (0-0.2mm from sawn surface (%+ve): 67%; 0-0.2mm from fracture surface: 16%; 0.2-0.4mm:- sawn: 68% fracture: 11%; 0.4-0.6mm:- sawn: 68%: fracture: 26%; 0.6-0.8mm:- sawn: 65%: fracture: 44%; 0.8-1.0mm:- sawn: 66%: fracture: 47%; 1.0-1.2mm:- sawn: 69%: fracture: 54%. Sclerostin expression slowly increased with increasing distance from the fracture site so that after 1.2mm it was similar to that seen close to the sawn surfaces. There was no clearcut loss of counterstained (sclerostin negative) osteocytes near the fracture surface.

In conclusion, local osteocytic sclerostin expression appeared markedly reduced within 0.5mm of the fracture surface. Given that these samples were taken within a few days of the fracture occurring this rapid loss of sclerostin expression occurs during the reactive (inflammatory) phase of fracture repair. Subsequently, this might assist recruitment and activation of osteoblasts, some from the local pre-osteoblast pool and others through re- activation of bone lining cells.

OC4

RESULTS OF BONE MORPHOGENETIC PROTEIN (BMP-7) ALONE OR IN COMBINATION WITH AUTOLOGOUS BONE GRAFT (ABG) FOR THE TREATMENT OF LONG BONE FRACTURE NON-UNIONS

A Shoaib*[1], MA Rashid[2], O Lahoti[3], AFG Groom[4], SL Phillips[5]

[1]Limb Reconstruction, Department of Orthopaedics, King's College Hospital, London, UK

Objectives:

Fracture non-union poses a significant challenge to treating orthopaedic surgeons. These patients often require multiple surgical procedures. The incidence of complications after Autologous Bone Graft (ABG) harvesting has been reported up to 44%. These complications include persistent severe donor site pain, infection, heterotopic ossification and antalgic gait. We retrospectively compared the use of BMP-7 alone in long bone fracture Non-union, with patients in whom BMP-7 was used in combination with the Autologous Bone Graft (ABG).

Material and Methods:

The databases of our dedicated Limb Reconstruction Unit were searched for patient with three common long bone fractures Non-unions (Tibia, Femur and Humerus). The patients who had intra-operative use of Bone Morphogenetic Protein (BMP-7) alone and in combination with ABG were evaluated. 53 Patients had combined use of ABG and BMP-7, and 65 patients had BMP-7 alone.

Results:

In the ABG and BMP-7 group, the union rate for femoral (n=18) Non-unions was 83%, for humeral (n=16) Non-unions 81%, and for tibia (n=19) Non-unions it was 47%. In the BMP-7 alone group, 83% of the femoral (n=12) Non-unions, 87% of the humeral (n=16) and 56% of the tibial (n=37) Non-unions healed. The common risk factors for Non-union were comparable in both the groups and included location and nature (open vs closed) of fracture, infection, smoking and NSAIDs use. The average time to union in ABG+BMP-7 group

was 8.1 months (range 3-30 months) and in BMP-7 alone group it was 7.2 months (range 3-24 months).
Conlusion:
Autologous Bone Grafting has a pivotal role in limb reconstruction surgery but its indication should be carefully evaluated in view of considerable morbidity associated with graft donor site. Our study did not show any significant difference in the union rates of common long bone fracture Non-unions treated with BMP-7 alone or with a combination of Autologous Bone Graft and BMP-7.

OC5
EFFECTS OF CHRONIC SYSTEMIC METAL EXPOSURE ON WHOLE BODY BONE MINERAL DENSITY AND BONE TURNOVER IN THE LATE PERIOD AFTER METAL-ON-METAL HIP RESURFACING
J Prentice*[1,2], M Clark[1,2], I Stockley[3], JM Wilkinson[1,2,3]
[1]Sheffield NIHR Bone Biomedical Research Unit, Sheffield;[2]Department of Human Metabolism, University of Sheffield;[3]Sheffield Teaching Hospitals NHS Foundation Trust
Background and objectives
Local bone-related adverse events occur more frequently following metal-on metal hip resurfacing (MOMHR) versus convention total hip arthroplasty (THA). High local tissue levels of cobalt and chromium may contribute to impaired bone health, however the systemic effects on bone of exposure to elevated metal levels after MOMHR are unknown.
Methods
In this cross-sectional study we compared whole body bone mineral density (WB-BMD) and biochemical markers of bone turnover in 31 healthy male subjects at a mean of 8 years after MOMHR versus 31 individually age and time since surgery matched male subjects after conventional THA. All subjects had well-functioning prostheses and were in good self-reported health as assessed by Oxford Hip Score and EQ-5D questionnaire. WB-BMD was measured by dual energy x-ray absorptiometry and adjusted for pre-morbid osteoporosis risk factors using the FRAX tool, and for the presence of the metal prostheses using identical exclusion regions. Bone turnover markers were measured on fasting morning serum or 24hr urine collection by electro-chemiluminescent assay. Cobalt and chromium were measured by ICP-MS.
Results
The subject pairs were similar for all matching criteria (P>0.05, all comparisons). Cobalt and chromium were elevated in the MOMHR versus THA group (P<0.05, all comparisons). WB-BMD was 7% higher in the MOMHR versus THA subjects (1.05 versus 0.98 g/cm2, P= 0.002). Bone formation, measured by serum osteocalcin, was 17% lower in the MOMHR versus THA subjects (18.2 versus 21.1ng/mL, P= 0.02) and osteoclast number measured by TRAP 5b, was 23% lower (3.4 versus 3.9U/L, P= 0.008). Systemic levels of bone formation and resorption markers were inversely correlated with systemic cobalt, but not chromium levels (Spearman, P<0.05).
Conclusion
Subjects 8 years after MOMHR versus THA have higher WB-BMD and lower bone turnover measured by some markers, and marker activity inversely correlate to systemic cobalt levels. These data suggest that this metal has a systemic suppressive effect on bone turnover. The difference in BMD may be due to a reduction in age-related bone loss since surgery, although the measured BMD might also be due, in part, to diffuse metal deposition within the skeleton.

OC6
HIGH FAILURE RATES WITH A LARGE DIAMETER HYBRID METAL ON METAL TOTAL HIP REPLACEMENT: CLINICAL, RADIOLOGICAL AND RETRIEVAL ANALYSIS
BJRF Bolland*[1], DJ Culliford[2], DJ Langton[3], JPS Millington[1], NK Arden[4,5], JM Latham[1].
[1]Southampton General Hospital, Southampton University Hospital Trust, Southampton; [2]Research Development Support Unit, University of Southampton; [3]University Hospital of North Tees, Newcastle University; [4]MRC ERC, University of Southampton;
[5]Biomedical Research Unit, University of Oxford.
This study reports the mid-term results of a large bearing hybrid metal on metal total hip replacement (MOMHTHR) in 199 hips (185 patients) with mean follow up of 62 months. Clinical, radiological outcome, metal ion levels and retrieval analysis were performed.
Seventeen patients (8.6%) had undergone revision, and a further fourteen are awaiting surgery (defined in combination as failures). Twenty one (68%) failures were females. All revisions and ten (71%) of those awaiting revision were symptomatic. Twenty four failures (86%) showed progressive radiological changes.
Fourteen revision cases showed evidence of adverse reactions to metal debris (ARMD). The failure cohort had significantly higher whole blood cobalt ion levels (p=0.001), but no significant difference in cup size (p=0.77), inclination (p=0.38) or cup version (p=0.12) in comparison to the non revised cohort. Female gender was associated with an increased risk of failure (chi squared p=0.04). Multifactorial analysis demonstrated isolated raised Co levels in the absence of either symptoms or XR changes was not predictive of failure (p=0.675). However both the presence of pain (p<0.001) and XR changes (p<0.001) in isolation were both significant predictors of failure. Wear analysis (n=5) demonstrated increased wear at the trunnion/head interface (mean out of roundness measurements of 34.5 microns +/-13.3 (+/-2SD, normal range 8-10 microns) with normal levels of wear at the articulating surfaces. There was evidence of corrosion at the proximal and distal stem surfaces. The cumulative survival rate, with revision for any reason was 92.4% (95%CI: 87.4-95.4) at 5 years. Including those awaiting surgery, the revision rate would be 15.1% with cumulative survival at 5 years of 89.6% (95% CI: 83.9-93.4).
This MOMHTHR series has demonstrated unacceptable high failure rates with evidence of high wear at the head/trunnion interface and passive corrosion to the stem surface. This raises concern with the use of large heads on conventional 12/14 tapers. Female gender was an independent risk factor of failure. Metal ion levels remain a useful aspect of the investigation work up but in isolation are not predictive of failure.

OC7
A BMPR1A SOLUBLE RECEPTOR CONSTRUCT INCREASES TIBIAL AND TRABECULAR BONE VOLUME IN VIVO
BAUD'HUIN M.*[1], LATH D. [1], CHANTRY AD. [1], SEEHRA J. [2], PEARSALL R.S. [2], BELLANTUONO I. [1], Croucher P.I. [1]
[1] Mellanby Centre for Bone Research, Faculty of Medicine Dentistry and Health, University of Sheffield, Sheffield, UK, [2] Acceleron Pharma, Cambridge, MA, USA.
Bone morphogenic proteins (BMPs) are members of the TGF-b superfamily and act as pleiotropic regulators of skeletal organogenesis and bone homeostasis. BMP signalling is regulated through a heterotetrameric receptor complex composed of both a BMP type I serine/threonine kinase receptor (including the BMP Receptor1A (BMPR1A), the BMP Receptor1B and the Activin Type Receptor 1) and a type II serine/threonine kinase kinase receptor. The various combinations of the Type I and Type II receptors results in modulation of ligand signalling on target organs. This has made defining the relative contribution of individual BMPs and TGF-b superfamily members regulating bone mass difficult. Therefore, the aim of this study was to determine the role of one component of this system, the BMPR1A, in regulating bone mass in vivo.
The extracellular domain of the murine BMPR1A fused to a murine Fc-domain was expressed in CHO cells to create a soluble BMPR1A fusion protein (RAP-661). C57BL/6 male mice were treated with RAP-661 (10mg/kg, twice a week, i.p.), or vehicle (PBS), and groups (n=6 to 9) sacrificed after 3, 7, 14 or 28 days. The tibia and vertebrae (L4) were analysed by microCT and processed for bone histomorphometry. Trabecular bone volume in the tibia was increased by 38%, 33% and 51% after 7, 14 and 28 days, respectively, in RAP661-treated mice compared to control (p<0.05 in each case). RAP-661 also increased trabecular bone volume in vertebrae by 18%, 34% and 55% at 7, 14 and 28 days (p<0.05 in each case). This was associated with an increase in trabecular number and trabecular thickness in both tibia (p<0.05) and vertebra at 7, 14, 28 days (p<0.05 and p<0.05). Histomorphometric analysis demonstrated an increase in osteoblast number at day 7 (p<0.05). There were no difference between RAP-661 treated mice and mice treated with vehicle at day 14 and 28. In contrast, osteoclasts number was not different from control at days 3 but were significantly decreased at day 14 and 28 (p<0.01).
These data demonstrate that inhibiting ligands that signalling through BMPR1A is able to increase bone mass and could offer a therapeutic approach to increasing bone mass.

OC8
THE MOLECULAR MECHANISM OF ISCHAEMIC PRECONDITIONING IN SKELETAL MUSCLE CELLS
Ciara McGuire* [1], Pauline Walsh [1], Prof. K.J. Mulhall [2]
[1] Catherine McAuley UCD Research Centre, Mater Misericordiae University Hospital, Dublin; [2] Department of Orthopaedics, Mater Misericordiae University Hospital, Dublin
OBJECTIVES: Ischaemic preconditioning (IPC) is a phenomenon whereby tissues develop an increased tolerance to ischaemia and subsequent reperfusion if first subjected to sublethal periods of ischaemia. Despite extensive investigation of IPC, the molecular mechanism remains largely unknown. Our aim was to show genetic changes that occur in skeletal muscle cells in response to IPC.
METHODS: Firstly, we established an in-vitro model of IPC using a human skeletal muscle cell line. Gene expression of both control and preconditioned cells at various time points was determined. The genes examined were HIF-1 alpha, EGR1, JUN, FOS, and DUSP1. HIF-1 alpha is a marker of hypoxia. EGR1, JUN, FOS and DUSP1 are early response genes and may play a role in the protective responses induced by IPC. Secondly, the expression of HSPB8 was examined in a cohort of preconditioned total knee arthroplasty patients.
RESULTS: HIF-1 alpha was upregulated following 1 and 2 hours of simulated ischaemia (p = 0.076 and 0.841 respectively) verifying that hypoxic conditions were met using our model. Expression of EGR1, FOS and DUSP1 were upregulated and peaked after 1 hour of hypoxia (p = 0.001, <0.00, and 0.038 respectively). cFOS was upregulated at 2 and 3 hours of hypoxia. IPC prior to simulated hypoxia resulted in a greater level of upregulation of EGR1, JUN and FOS genes (p = <0.00, 0.047, and <0.00 respectively). HSPB8 was not significantly upregulated following IPC using the hypoxic model. It was,

however, upregulated on an mRNA level in total knee arthroplasty patients (p = 0.15).
CONCLUSION: This study has validated the use of our hypoxic model for studying IPC in-vitro. IPC results in a greater upregulation of protective genes in skeletal muscle cells exposed to hypoxia than in control cells. We have demonstrated hitherto unknown molecular mechanisms of IPC in cell culture and in patients undergoing TKA.

OC9
ACCELERATED SKELETAL DEVELOPMENT AND HIGH BONE MASS IN MICE LACKING RAMP3
S Pacharne*[1], G Richards[1], K Caron[2] and T Skerry[1]
[1]The Mellanby Centre for Bone Research. The Medical School, The University
of Sheffield , Beech Hill Rd, Sheffield ,S10 2RX; [2]Department of Cell and Molecular Physiology, CB # 7545,6340B MBRB, 103 Mason Farm Road, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599. USA
Receptor Activity Modifying proteins (RAMPs) are important accessory proteins for G- protein coupled receptors (GPCRs). RAMP isoforms (RAMP1, 2 & 3), alter ligand selectivity, trafficking of receptors and regulate subtle aspects of G-protein activation in a range of partner GPCRs.
The predominant roles of RAMPs in regulation of ligand selectivity occur in receptors for Calcitonin (CT) and its related family of peptides: Calcitonin gene related peptide (CGRP), Amylin (AMY) and Adrenomedullin (AM) which are important in skeletal development and maintenance. Functional receptors to these peptides comprise heterodimers of a RAMP with either the CT receptor (CTR) or Calcitonin- like receptor (CLR).
Published evidence has demonstrated the bone anabolic effects of AM and AMY in vitro and in vivo. We hypothesised that altering RAMP expression would change skeletal phenotype. We analysed bones from mouse pups (5 days postnatal) and smaller numbers of adult mice, lacking expression of RAMPs 1&3. As -/- RAMP2 mice are not viable, we analysed heterozygotes, and in each case compared data with age matched controls on the same genetic background.
RAMP1 KO mice had no significant differences from WT controls. In contrast, RAMP3 pups stained with alizarin red/alcian blue had evidence of accelerated development in the axial skeleton with larger vertebral bodies and lateral transverse processes in the lumbar vertebrae. In the hind limb, femoral bone volume measured by microCT was increased by 40% compared with WT controls. In small numbers of adult mice (8 weeks old) microCT analysis of femurs of RAMP3 KO mice displayed increased trabecular thickness/reduced trabecular spacing and a marked increase in cortical thickness that was approximately double the WT controls. RAMP2 heterozygote mice have a haploid insufficiency phenotype with clear endocrine disturbances. Bones of adult RAMP2+/- mice had thinner cortices than WT controls, and evidence of increased intracortical porosity.
These data suggest that RAMPs 2&3 have the ability to regulate skeletal mass. Whether this is due to roles in regulating AM and AMY functions, or through effects on other osteotropic hormone receptors which interact with RAMPs is not yet clear. However, modulation of RAMP function may offer opportunities for anabolic therapies.

OC10
PAGET'S DISEASE-CAUSING MUTATIONS IN SEQUESTOSOME-1 AFFECT AUTOPHAGIC PROTEIN DEGRADATION
E Azzam*, A Duthie, MH Helfrich, LJ Hocking
Musculoskeletal Research Programme, University of Aberdeen, UK
Paget's disease of bone (PDB) is characterised by focal lesions of increased bone turnover, driven by overactive osteoclasts. Mutations affecting Sequestosome-1 (SQSTM1/p62) ubiquitin-associated domain have been identified in individuals with PDB, which impair binding to ubiquitylated proteins targeted for degradation. We have previously shown elevated abundance of SQSTM1, ubiquitin and proteasomal subunits in osteoclasts from Pagetic biopsies, suggesting defects in proteasomal degradation. The aim of this study was to examine the complementary process of autophagic degradation, and to assess the effect of PDB-causing SQSTM1 mutations.
Bone biopsies from four PDB patients without SQSTM1 mutations and four non-Pagetic controls were immunostained for LC3 (autophagy marker; Bond Autostainer). Osteoclast LC3 was quantified using Volocity image analysis software and differences assessed by t-test. HEK293 cell lines stably expressing exogenous wild-type and mutated SQSTM1 (P392L, E396X and G425R) were generated using the Flp-In System (Invitrogen). These cells also express endogenous SQSTM1, approximating heterozygous mutations. Cells were examined under normal growth conditions and following amino acid starvation to induce autophagy; lysates were western blotted for SQSTM1, LC3 and actin. Protein abundance was measured using LiCOR Odyssey and results expressed relative to actin, normalised to the parent cell line (endogenous SQSTM1, LC3) or wild-type SQSTM1 cell line (exogenous SQSTM1). Differences between cell lines were assessed by Kruskal-Wallis test (n=3).
In bone biopsies, LC3 abundance was increased in Pagetic osteoclasts compared to controls (p= 0.04), suggesting autophagy is induced above basal levels. Between cell lines, exogenous SQSTM1 abundance differed under normal conditions (p=0.02) as well as upon starvation (p=0.02), suggesting SQSTM1 degradation is affected by PDB mutations. Endogenous SQSTM1 abundance differed between cell lines upon starvation (p=0.03) but not under normal conditions. Autophagosome-associated LC3 also differed between cell lines (p(normal)=0.03; p(starvation)=0.04). Effects varied between mutations, with missense mutations but not E396X displaying increased autophagic flux.
We have shown that autophagic protein degradation is altered in osteoclasts from patients with PDB, and is affected by PDB-causing SQSTM1 mutations. Pharmacological dissection of protein degradation dynamics will provide important insights into the regulation of osteoclast function by protein degradation pathways and the influence of SQSTM1 mutations on this.

OC11
LACK OF NUCLEOTIDE PYROPHOSPHATASE PHOSPHODIESTERASE1 ACTIVITY IN Enpp1-/- MICE RESULTS IN ALTERED BONE DEVELOPMENT AND PATHOLOGICAL MINERALISATION OF SOFT TISSUE
NCW Mackenzie*[1], D Zhu[1], EM Milne [1], JL Millan[2], C Farquharson[1], VE MacRae[1]
[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, UK; [2]Sanford-Burnam Medical Research Institute, La Jolla, California, USA
Nucleotide pyrophosphatase phosphodiesterase 1 (NPP1) is required to convert extracellular ATP into inorganic pyrophosphate (PPi), a crucial negative regulator of hydroxyapatite (HA) crystal formation. NPP1, together with bone alkaline phosphatase, is responsible for maintaining the balance between PPi and inorganic phosphate (Pi) and thereby regulating the rate of HA formation in the developing skeleton. In this present study a detailed phenotypic assessment of a mouse model lacking NPP1 (Enpp1-/-) was carried out to further the understanding of its role in skeletal and soft tissue mineralisation. Histopathological assessment of Enpp1-/- mice at 22 weeks of age revealed calcification in the aorta and kidney and ectopic cartilage formation in the joints and spine. Radiographic assessment of the hind limb showed evidence of hyper-mineralisation in the talocrural joint and hypo-mineralisation in the femur and tibia. Comprehensive microCT analysis of the tibia and femur examined the effects of Enpp1 ablation on trabecular architecture and bone geometry at 6 and 22 weeks of age in both male and female mice. Trabecular number, trabecular percentage bone volume, structure model index, trabecular and cortical thickness were significantly reduced ($P<0.05$) in tibiae and femurs from the Enpp1-/- mice compared to wild-type controls. This decreased bone mass was reflected by a significant reduction in maximum bone stiffness in Enpp1-/- tibiae and femurs from 22 week old mice ($P<0.05$) as determined by three-point bending. Circulating phosphate and calcium plasma levels were also reduced ($P<0.05$) in the Enpp1-/- null mice. Plasma levels of osteocalcin, a marker of bone formation, were significantly decreased at 6 weeks of age ($P<0.05$) in Enpp1-/- mice, with no differences noted at 22 weeks of age. Plasma levels of CTx (RatlapsTM), a marker of bone resorption, and the phosphaturic hormone FGF-23 were significantly increased in the Enpp1-/- mice at 22 weeks of age ($P<0.05$). These results demonstrate that Enpp1-/- mice are characterised by severe disruption to the architecture and mineralisation of long-bones, dysregulation of calcium/phosphate homeostasis and hypercalcification in soft tissues. In summary, NPP1 is essential for normal bone development and the control of physiological bone mineralisation and pathological mineralisation in soft tissues.

OC12
METAL ION LEVELS AND CHROMOSOME ABERRATIONS IN METAL-ON-METAL (MoM) AND METAL-ON-POLYETHYLENE (MoP) TOTAL HIP ARTHROPLASTY
BS Dhinsa[1], JR Perera*[1], KR Gallagher[1], B Spiegelberg[1], S Hanna[1], S Tai[1], R Pollock[1], R Carrington[1], SR Cannon[1] and TWR Briggs[1]
[1]Royal National Orthopaedic Hospital NHS Trust, UK
The aim of this study is to investigate whether MoM implants result in more chromosome aberrations and increased blood metal ions postoperatively whe compared to MoP implants.
MoM arthroplasties are being inserted in increasing numbers of younger patients due to the increased durability and reduced requirements for revision in these implants. Recent studies have raised many concerns over possible genotoxicity of MoM implants.
This is a prospective study of patients who have undergone elective total hip replacement, they were selected and then randomised into two groups. Group A received a MoP implant and group B received a MoM implant. Patients are reviewed pre-operatively (control group), at 3 months, 6 months, 1 year and 2 years post-operatively. On each occasion blood tests are taken to quantify metal ion levels (chromium, cobalt, titanium, nickel and vanadium) using HR-ICPMS method and chromosome aberrations in T lymphocytes using 24 colour fluorescent in situ hydridisation (FISH).
51 patients have been recruited to date, 23 of whom had MoP prosthesis and 28 a MoM. 47 of these had their 1 year follow-up with blood analysis and 38 have had 2 year follow up. There appeared to be a bedding period for both MoM and MoP groups, with an increase in metal ion release. The blood concentration of

chromium, cobalt and titanium rise significantly in the MoM group at the 2 year stage. Chromosome aberrations occurred in both groups. Both the MoM and MoP groups showed increase frequency of aneuploidy aberrations and structural damage. The greatest increase in metal ion levels occurred at the 1 to 2 year interval corresponding to significant rise in chromosome aberrations.
Preliminary results of this study show that the levels of chromium, cobalt and titanium are significantly higher in the MoM group compared to the MoP group. This corresponds to increases in chromosome aberrations in the groups with increases in structural chromosome damage after two years.

OC13
PLATELET RICH PLASMA IN ACCELERATED ACHILLES TENDON REGENERATION: A RANDOMIZED CONTROLLED TRIAL
J Alsousou*, R Handley, P Hulley, M Thompson, E McNally, P Harrison, K Willett
Oxford, UK
Platelet Rich Plasma (PRP) has been shown to have positive effect in tendon regeneration in in-vitro and limited in-vivo animal studies. We aim to study PRP use in acute Achilles tendon rupture (ATR) regeneration in a purposely designed clinical trial.
This is a prospective double-arm patient-blinded randomized controlled trial. ATR patients were randomized into PRP treatment or control groups. Non-operatively treated patients received PRP or control injection in clinic. In operatively treated patients, PRP gel was applied in the ruptured gap during percutaneous repair. Standard rehabilitation protocol was used and patients were followed up for 24 weeks. ATR, VISA-A and FAOS scores were used as subjective outcome measures. Functional ultrasound Elastography (FUSE) was performed at each follow-up to assess the mechanical properties of tendons. PRP analysis and tendon needle-biopsy were performed to study the histological differences during healing in both groups.
20 patients were recruited with mean age 37.5±8.8 (8males and 7 females). Rupture location was 4.8±2.1 cm from insertion. PRP platelet count 1044±320 x1000/µL with average platelet CD62p activation 68.42±4.5%. Mixed linear regression analysis revealed PRP treated tendon achieved better ATR and VISA-A outcome scores ($p<0.05$). FAOS score analysis showed that PRP group had better pain, ADL and symptoms scores with significant difference apparent from week 3 onwards. Strain mapping using FUSE scan in 4 patients showed bigger harder tendons in PRP group. Analysis of the remaining patients is on the way. To achieve the desired statistical power in pragmatic settings, recruitment will continue in a multi-centre trial.
Our preliminary findings show that PRP application in Achilles tendon rupture may lead to faster regeneration and return to function as supported by a combination of objective and subjective outcome measures.

OC14
ENRICHMENT OF SKELETAL STEM CELLS FROM BONE MARROW TO ENHANCE SKELETAL REGENERATION - A NOVEL CLINICAL TECHNIQUE
JO Smith*[1], JI Dawson[1], A Aarvold[1], AMH Jones[1], JN Ridgway[2], SJ Curran[2], DG Dunlop[1], ROC Oreffo[1]
[1]Bone and Joint Research Group, University of Southampton, UK;[2]Smith and Nephew Research Centre, York, UK
Recent approaches have sought to harness the potential of stem cells to regenerate bone lost as a consequence of trauma or disease. Bone marrow aspirate (BMA) provides an autologous source of skeletal stem cells (SSCs) for such applications, however previous studies have demonstrated that the concentration of SSCs present in iliac crest BMA is below that required for robust bone regeneration. Here we present a novel acoustic-facilitated filtration strategy to concentrate BMA for SSCs, clinically applicable for intra-operative orthopaedic use.
The aim of this study was to demonstrate the efficacy of this strategy in concentrating SSCs from iliac crest bone marrow, as well as femoral canal BMA from older patients.
Iliac crest BMA (Lonza, Rockville, MD, USA) and femoral canal BMA was obtained with informed consent from older patients during total hip replacement. 5 to 40ml of BMA was processed via the acoustically-aided exclusion filtration process to obtain 2-8 fold volume reductions. SSC concentration and function was assessed by flow-cytometry, assays for fibroblastic colony-forming units (CFU-F) and multi-lineage differentiation along chondrogenic, osteogenic and adipogenic pathways examined. Seeding efficiency of enriched and unprocessed BMA (normalised to cell number) onto allograft was assessed.
Iliac crest BMA from 15 patients was enriched for SSCs in a processing time of only 15 minutes. Femoral BMA from 15 patients in the elderly cohort was concentrated up to 5-fold with a corresponding enrichment of viable and functional SSCs, confirmed by flow cytometry and assays for CFU-F. Enhanced osteogenic ($P<0.05$) and chondrogenic ($P<0.001$) differentiation was observed using concentrated aspirate, as evidenced by biochemical assay and semi-quantitative histological analysis. Furthermore, enhanced cell seeding efficiency onto allograft was seen as an effect of SSC concentration per ml of aspirate ($P<0.001$), confirming the utility of this approach for application to bone regeneration.
The ability to rapidly enrich BMA demonstrates potential for intra-operative application to enhance bone healing and offers immediate capacity for clinical application to treat many scenarios associated with local bone stock loss. Further in vivo analysis is ongoing prior to clinical tests.

OC15
THE SYSTEMIC STIMULATION OF MESENCHYMAL STEM CELLS (MSCS) IN BONE MARROW IN RESPONSE TO TRAUMA
HB Tan*, E Jones, L Kozera, K Henshaw, D McGonagle, PV Giannoudis
Academic Department of Trauma & Orthopaedics, University of Leeds
Background and objectives
Fracture healing represents a physiological process regulated by a variety of signalling molecules, growth factors and osteogenic progenitor cells. Bone healing following trauma is associated with increased serum concentrations of several pro-inflammatory and angiogenic growth factors1. Platelet-derived growth factor (PDGF) has been shown to stimulate mesenchymal stem cell (MSC) proliferation in vitro. However, the in vivo relationship between the levels of PDGF and the numbers of MSCs in humans has not yet been explored. The aim of this study was to investigate PDGF release in the peripheral circulation following trauma and to correlate it with the numbers of MSCs in iliac crest bone marrow (BM) aspirate and in peripheral blood.
Methods
Trauma patients with lower extremity fractures (n=12, age 18-63 years) were recruited prospectively. Peripheral blood was obtained on admission, and at 1, 3, 5 and 7 days following admission. The serum was collected and PDGF was measured using the enzyme-linked immuno-sorbent assay (ELISA) technique. Iliac crest (BM) aspirate (20ml) and peripheral blood (PB) (20ml) was obtained on days 0-9 following admission. MSCs were enumerated using standard colony-forming unit fibroblasts (CFU-F) assay.
Results
We observed a gradual increase in serum PDGF levels following fracture ($r2=0.79$, $p=0.005$, $n=8$), which reached up to 2-fold on day 7. In 5 out of 8 patients recruited for CFU-F study, an increase in iliac crest BM CFU-F per millilitre of aspirate was similarly observed, which reached an average 6-fold post-fracture (ranging from day 3 to day 9). No CFU-Fs were observed in PB at any time-point in all patients studied. In three patients, for which PDGF and CFU-F were measured in parallel, a strong positive correlation was observed between CFU-F numbers per millilitre of BM aspirate and circulating PDGF levels ($r2=0.98$, $p<0.01$).
Conclusion
Our data demonstrate, for the first time, that BM MSC pool in humans is not static and can be stimulated following trauma. This is not a result of mobilisation of MSCs into systemic circulation. Rather, MSC activation at remote sites, like iliac crest BM, can be due to systemic up-regulation of several cytokines and growth factors, including PDGF, in peripheral circulation.

OC16
HOW DO OSTEOCLASTS GENERATE ENERGY TO MAINTAIN BONE RESORPTION WITHIN A HYPOXIC ENVIRONMENT?
HJ Knowles*, NA Athanasou
Botnar Research Centre, NDORMS, University of Oxford, Oxford, UK
Bone resorption is an energy-intensive process mediated by osteoclasts, the activity of which increases in a hypoxic environment (Knowles & Athanasou, J Pathol 218:256; 2009). Osteoclasts exhibit increased citric acid cycle and mitochondrial respiration rates in order to generate ATP for the energy-intensive resorption process (Kim et al, Cell Physiol Biochem 20:935, 2007). We have investigated whether osteoclasts are able to meet their hypoxic energy requirements by switching to anaerobic glycolysis, as is the case for monocytes / macrophages and other cells of the haematopoietic lineage (Cramer et al, Cell 112:645; 2003).
Osteoclasts were differentiated from CD14+ PBMC with M-CSF (25 ng/ml) and RANKL (50 ng/ml) for 16 days. An Illumina HumanWG-6 v3.0 48k array performed on 6 paired samples of normoxic versus hypoxic (2% O2, 24 h) osteoclasts identified hypoxic induction of a range of glycolytic enzymes (e.g. LDHA, PGK1, PFKFB3, ENO2), subsequently confirmed by real-time PCR. Hypoxic over-expression of the glucose transporter, Glut-1, was also evident at both the mRNA and protein level. Hypoxia resulted in a 2.1-fold increase in glucose consumption ($p<0.05$), a 2.3-fold increase in lactate production ($p<0.001$) and a 2.1-fold increase in complex I activity ($p<0.05$). Exposure of mature osteoclasts to the glycolytic inhibitor 2-deoxy-d-glucose resulted in significant cell death after 24h (22% reduction, 10mM 2DG, $p<0.01$), whereas the same concentration of glucose increased osteoclast numbers 2-fold ($p<0.001$). Compared with either their monocytic precursors or osteoblastic cells (no change in cell number), osteoclasts exhibited extreme sensitivity to hypoxia-induced cell death (68% reduction in cell number after 72h at 2% O2, $p<0.001$).
The lack of hypoxic effect on the 'lactate production':'glucose consumption' ratio and increased activity within the mitochondrial electron transport chain suggests that osteoclasts do not switch to anaerobic glycolysis, but that hypoxia instead initiates an increase in activity throughout the respiratory chain. Increased glucose utilisation within a low energy environment will rapidly deplete local substrate stores, possibly explaining osteoclast sensitivity to

hypoxia-induced cell death. These results suggest a potential mechanism whereby osteoclasts are able to generate sufficient energy, in the short term, to mediate pathological levels of bone resorption within a hypoxic environment.

OC17

FLUID FLOW STIMULATES ATP RELEASE FROM HUMAN DERIVED OSTEOCLASTS WITHOUT CHANGING RESORPTION

RMH Rumney*, A Agrawal, K Shah, A Gartland

Mellanby Centre for Bone Research, Department of Human Metabolism, The University of Sheffield, Beech Hill Road, Sheffield, S10 2RX, United Kingdom

Purinergic signalling, whereby extracellular nucleotides act upon cell surface P1 and P2 receptors, is an important mediator of bone remodelling. Both osteoblasts and osteoclasts express multiple P2 receptors and osteoblasts are known to release ATP in response to mechanical loading. No previous study has investigated ATP release or response to loading in human osteoclasts. The aim of this study was to determine whether human osteoclasts release ATP, whether ATP release is modulated by mechanical loading and what would be the subsequent effect on osteoclast activity.

Human peripheral blood monocytes were cultured on dentine discs or coverslips in the presence of RANKL and M-CSF. On day 14, cultures were subjected to up to mechanical loading in the form of 80% fluid displacement by pipette. Half of all cultures were fixed on day 14 with the remainder kept for up to 21 days. All cultures were stained for Tartrate resistant acid phosphatase (TRAP) to quantify osteoclast number and resorption.

A significant increase in ATP release was observed from osteoclasts in response to 5 bouts of mechanical loading both on coverslips and dentine (42.6nM, $P<0.001$ cf basal and 114.2nM, $P<0.01$ cf basal respectively). Interestingly, when osteoclasts were cultured on dentine they released significantly more ATP in response to loading compared to when cultured on glass, and also significant ATP release in response to fewer bouts of loading (56.8nM, $P<0.01$). TRAP staining demonstrated there was no significant correlation with number of osteoclasts and basal ATP release, however there was an inverse correlation with basal ATP release and the amount of resorption on day 14 ($P<0.001$). Surprisingly, load-induced ATP release did not have a significant effect on resorption at 21 day. These findings demonstrate for the first time that osteoclasts respond to mechanical loading in the form of fluid flow by releasing ATP, and that active osteoclasts have an enhanced sensitivity to fluid flow.

OC18

OSTEOBLAST EXTRACELLULAR MATRIX MINERALIZATION IS PROMOTED BY PHOSPHO1 OVEREXPRESSION

Huesa C*[1], Yadav M[2], Seawright E[1], Millan JL[2], Farquharson C[1]

[1]Department of developmental genetics, Roslin Institute, University of Edinburgh, UK;[2]Sanford-Burnham Medical Research Insititute, La Jolla, CA, USA.

During the process of bone formation, osteoblasts mineralize their extracellular matrix (ECM) by promoting the initial formation of crystalline hydroxyapatite (HA) inside matrix vesicles (MV). Bone alkaline phosphatase (TNAP) is important for ECM mineralization yet TNAP null (Akp2-/-) mice are born with a normal mineralized skeleton. The phosphatase PHOSPHO1 is highly expressed by osteoblasts and chondrocytes and its developmental expression profile coincides with the initial stages of mineralization. Membrane phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC) have been identified as putative substrates for PHOSPHO1. The skeleton of Phospho1-/- mice is characterized by osteoidosis, which results in altered material and mechanical properties, greenstick fractures and scoliosis. In this study we examined the effects of PHOSPHO1 overexpression in osteoblast-like cells in the presence of various substrates to further characterize the function of this phosphatase in the mineralization process. Two subclones of the MC3T3-E1 cell line previously characterized as mineralizing (clone 14) and non-mineralizing (clone 24) were used. Over a 21-day culture, ECM mineralization by clone 14 was first noted at day 11 when beta-glycerol phosphate (BGP) and PC were substrates. No mineralization was noted with PE. ECM mineralization of clone 24 was less than that noted in clone 14 and was first noted at day 11 (BGP), 14 (PC) and 21 (PEA). Clone 14 expressed 10 times more PHOSPHO1 protein than clone 24, which may explain the greater ECM mineralization noted in clone 14. A lentiviral vector was constructed to induce the overexpression of mouse recombinant PHOSPHO1. An empty vector was used as control. PHOSPHO1 overexpression in clone 14 resulted in a 20% increase in PHOSPHO1 protein levels and no alteration in ECM mineralization at day 14 in the presence of BGP, PE or PC. PHOSPHO1 overexpression in clone 24 showed an 80% increase in PHOSPHO1 expression when compared to the control cells and a 10-fold increase in mineralization with BGP, PE and PC as substrate. These data highlight the importance of PHOSPHO1 in the control of osteoblast ECM mineralization and work is in progress to uncover the mechanisms of action of this phosphatase and its functional interaction with TNAP.

OC19

IN VIVO EVALUATION OF MINERALISED COLLAGEN-GLYCOSAMINOGLYCAN AS A BONE GRAFT SUBSTITUTE

MQ Arumugam*[1], AK Lynn[2], N Rushton[1], RA Brooks[1]

[1]Orthopaedic Research Unit, University of Cambridge;[2]Tigenix

Porous collagen-glycosaminoglycan (Col/GAG) scaffolds have previously been used clinically as regeneration templates for peripheral nerves and skin [1]. For defects involving even minimal load-bearing applications however, these scaffolds do not possess the required stiffness. Calcium phosphates (CaPs) are often used as bone-graft substitutes due to their biocompatibility and direct bone-bonding ability. While CaPs have sufficient stiffness for bone-defect applications, unlike Col/GAG they lack elasticity and are very brittle. Combining these two materials produces a composite with enhanced material properties and chemical similarity to natural bone. The addition of CaP nanocrystallites into the Col/GAG matrix produces a 3-dimensional structure that maintains its structural integrity even when wet. In this study, the in vivo performance of mineralised Col/GAG composites was evaluated by implantation into a six-week ovine bone-defect model.

Four different materials were implanted; Col/GAG alone, Col/GAG with octacalcium phosphate, Col/GAG with hydroxyapatite and Col/GAG with brushite. Implants with a diameter of 9mm and length of 9mm, were placed bilaterally into the distal femoral condyle of the hind legs of thirteen sheep. This site was selected due to the large volume of load-bearing cancellous bone. Cancellous autograft was harvested from the tibial tuberosity and placed in the defect sites of two sheep as a positive control.

All animals were sacrificed after 6 weeks and tissue containing the implants was prepared for histological evaluation. Image analysis of Von Kossa stained sections showed that all mineralised Col/GAG implants had significantly more bone in the implant site than unmineralised Col/GAG but were not significantly different between CaPs. Interestingly, new bone formation often followed the structure of the porous material struts which acted as a template. The defect containing the autograft contained the greatest amount of new bone.

Conclusions

o The inclusion of mineral substantially improves the osteoconductivity of Col/GAG.

o No significant difference between the different calcium phosphates was seen.

o Whilst these materials did not stimulate bone formation to the same extent as autograft, many bone graft procedures are carried out with allograft which performs less favourably.

[1] Yannas, Burke et al, J Biomed Mater Res, 14 (2), 1980

OC20

INTRAOSSEOUS TRANSCUTANEOUS AMPUTATION PROSTHESES VS DENTAL IMPLANTS: A COMPARISON BETWEEN KERATINOCYTE AND GINGIVAL EPITHELIAL CELL ADHESION IN VITRO

CJ Pendegrass*[1], C Fontaine[1], GW Blunn GW[1]

[1]University College London, The Institute or Orthopaedics & Musculoskeletal Science, Centre for Biomedical Engineering, The RNOH, Brockley Hill, Stanmore, Middlesex, HA7 4LP

Infection is the primary failure modality for transcutaneous implants because the skin breach provides a route for pathogens to enter the body. Intraosseous transcutaneous amputation prostheses (ITAP) are being developed to overcome this problem by creating a seal at the skin-implant interface to prevent bacterial invasion. Oral gingival epithelial cell adhesion creates an infection free seal around dental implants; however this has yet to be demonstrated outside the oral environment. All epithelial cells attach via hemidesmosomes (HD) and focal adhesions (FA) and their expression is an indicator of adhesion efficiency. The aim of this study was to compare epidermal keratinocyte with oral gingival epithelial cell adhesion on titanium alloy in vitro to determine whether these two cell types differ in their speed and strength of adhesion. It was hypothesised that oral gingival epithelial cells attach to titanium alloy earlier than epidermal keratinocytes; with greater expression of hemidesmosomes and focal adhesions.

Human oral gingival epithelial cell (HGEP) and primary human epidermal keratinocyte (HPEK) adhesion to titanium alloy, was assessed at 4, 24, 48 and 72 hrs. Adhesion was measured by the number of FAs per unit cell area and expression of HDs using a semi-quantitative scale.

At 4 and 24hrs, there was a significant increase in vinculin marker expression per unit cell area of 4.3 and 4.7 times in HGEP compared with HPEK ($p=0.000$). At 48 and 72hrs there were no significant differences.

HD expression was significantly greater in HGEP at 4 and 24hrs ($p=0.002$) compared with HPEK. Up-regulation of HD expression in HPEK lagged that of HGEP until 48hrs, after which no significant differences were observed.

This study has demonstrated that oral gingival cells up-regulate both focal adhesion and hemidesmosome expression at earlier time points compared with epidermal keratinocytes. Expression of hemidesmosomes lags that of focal adhesions, suggesting that focal adhesion formation is a prerequisite for hemidesmosome assembly. We postulate that early attachment of oral gingival epithelial cells to dental implant biomaterials may be responsible for the formation of an infection-free seal.

OC21

SEPTIC ARTHRITIS VS TRANSIENT SYNOVITIS IN CHILDREN: A TERTIARY HEALTH CARE CENTRE STUDY

R Singhal*[1],D Perry[2],FN Khan[2],D Cohen[2],HL Stevenson[2],LA James[2],JS Sampath[3],CE Bruce[2]

[1]Macclesfield District General Hospital UK,[2]Alder Hey Children's Hospital UK,[3]Sparsh Hospital India

Background: Establishing the diagnosis in a child presenting with an atraumatic limp can be challenging. There is particular difficulty distinguishing septic arthritis (SA) from transient synovitis (TS) and consequently clinical prediction algorithms have been devised to differentiate the conditions using the presence of fever, raised erythrocyte sedimentation rate (ESR), raised white cell count (WCC) and inability to weight bear. Within Europe measurement of the ESR has largely been replaced with assessment of C-reactive protein (CRP) as an acute phase protein. We have evaluated the utility of including CRP in a clinical prediction algorithm to distinguish TS from SA.

Method: All children with a presentation of 'atraumatic limp' and a proven effusion on hip ultrasound between 2004 and 2009 were included. Patient demographics, details of the clinical presentation and laboratory investigations were documented to identify a response to each of four variables (Weight bearing status, WCC >12,000 cells/m3, CRP >20mg/L and Temperature >38.5 degrees C. The definition of SA was based upon microscopy and culture of the joint fluid collected at arthrotomy.

Results: 311 hips were included within the study. Of these 282 were considered to have transient synovitis. 29 patients met criteria to be classified as SA based upon laboratory assessment of the synovial fluid. The introduction of CRP eliminated the need for a four variable model as the use of two variables (CRP and weight bearing status) had similar efficacy. An algorithm that indicated a diagnosis of SA in individuals who could not weight-bear and who had a CRP >20mg/L correctly classified SA in 94.8% individuals, with a sensitivity of 75.9%, specificity of 96.8%, positive predictive value of 71.0%, and negative predictive value of 97.5%. CRP was a significant independent predictor of septic arthritis.

Conclusions: CRP was a strong independent risk factor of septic arthritis, and its inclusion within a regression model simplifies the diagnostic algorithm, such that a two-variable model correctly classified 95% individuals with SA. Nevertheless, this and similar algorithms are generally more reliable in excluding SA, than confirming SA, and therefore a clinician's acumen remains important in identifying SA in those individuals with a single abnormal variable.

OC22

HISTOLOGICAL RESULTS OF 406 BIOPSIES FOLLOWING ACI/MACI PROCEDURES FOR OSTEOCHONDRAL DEFECTS IN THE KNEE

AS Shekkeris*[1], JR Perera[1], G Bentley[1], AM Flanagan[1], J Miles[1], RWJ Carrington[1], JA Skinner[1], TWR Briggs[1]
[1]Royal National Orthopaedic Hospital Stanmore, Middlesex

Articular cartilage implantation (ACI) and associated procedures (MACI = Matrix-assisted cartilage implantation) are now established treatments for osteochondral defects in the knee. The quality of repair in terms of histological appearance is frequently not known, whilst the correlation of histology results with functional outcomes remains undefined. Histological data of the quality of the repair tissue is sparse and a precise classification proved difficult.

This was a single-centre, prospective study. Over 12 years (1998-2010) 406 patients that underwent articular cartilage implantation procedures at our institution (ACI = 170, MACI = 205) had biopsies taken at the 1-2 year interval, in order to assess whether these contained 'hyaline-like' cartilage, 'mixed hyaline-like with fibrocartilage', fibrocartilage or fibrous tissue alone.

Histological sections of the biopsies were prepared and stained with haematoxylin, eosin and proteoglycan stains and viewed under polarised light. All biopsies were studied by a single histopathologist in a specialist, dedicated musculoskeletal laboratory.

All patients were assessed by the Cincinnati, Bentley and Visual Analogue scores both pre-operatively and at the time of the review.

The findings revealed that 56 patients healed with 'hyaline-like' cartilage (14.9%), 103 with 'mixed' (27.5%), 179 with fibrocartilage (47.7%) and 37 with fibrous tissue (9.9%).

These findings showed that 42.4% of defects were filled with 'hyaline-like' or 'mixed' cartilage, with 70% of these achieving a 'fair' to 'excellent' functional outcome. This was also observed in the fibrocartilage group, where 72% achieved similar results. Predictably 89% of the patients that healed by fibrous tissue had a poor functional outcome.

This study shows that 71% of patients whose osteochondral defects healed by either 'hyaline-like', 'mixed' or fibrocartilage experienced an improvement in the function. In contrast, only 11% of the patients whose defects filled with fibrous tissue, showed some functional improvement. Additionally, this data indicates the advantage of biopsies in assessing the overall results of cartilage implantation procedures.

OC23

INVESTIGATING THE INTERACTION OF RAT OSTEOBLASTS WITH TITANIUM OXIDE NANOLAYERS

SR Deshmukh*[1], MA Birch[1], D Robbins[2], AW McCaskie[1]
[1] Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University, UK; [2] The Centre for Process Innovation Ltd, School of Electrical, Electronic and Computer Engineering, Merz Court, Newcastle University, UK

We used an atomic layer deposition (ALD) approach to create titanium oxide nanolayers on ultra high molecular weight polyethylene (UHMWPE) surfaces. These materials were then characterised in terms of rat osteoblast adhesion, morphology and differentiation.

UHMWPE discs produced from a machined cylinder or impact moulded discs were coated with titanium oxide by ALD. Light, atomic force microscopy and scanning electron microscopy with EDX were used to characterise the coated surfaces. These approaches showed 1-1.5 micron tooling grooves with a periodicity of 40 microns on the machined discs whilst the moulded discs exhibited nanotopographical features. The titanium oxide coating was successfully deposited on discs from both sources but was not uniform across the surfaces, with vein-like 'creases' clearly visible. We believe that these features are due to the thermal expansion of the UHMWPE discs during the ALD process and their subsequent cooling.

Coated and uncoated discs were seeded with osteoblasts for 24 hours, then fixed. Immunofluorescence microscopy and computer-based image processing enabled determination of osteoblast numbers, size and shape. A trend of larger average cell area was associated with the coated discs and $P<0.01$ for an H0 of no difference in cell area between coated and uncoated grooved discs.

Osteoblasts were also cultured on the discs in osteogenic medium to promote bone nodule formation. After a few weeks, von Kossa staining and computer-based image processing allowed calculation of surface area covered with bone nodules for each of the discs. Based on results from three of each type of disc, a significantly greater proportion of the surface area of coated discs was covered with calcified deposits compared to uncoated discs ($P<0.025$ for grooved discs and $P<0.005$ for smooth discs). On average, the coated discs had bone nodules on 1.4 times the surface area as compared to their uncoated counterparts.

The hypothesis for our study was that TiO2 coating of a polymer might better promote osteoblast interaction with the biomaterial surface leading to enhanced osteogenesis. Our preliminary data support this view and suggest that this approach could likely be exploited in the fabrication of implant materials with tailored biological activity.

OC24

MEPE REGULATES GROWTH PLATE MINERALISATION THROUGH ITS CLEAVAGE TO THE ASARM PEPTIDE

KA Staines*[1], VE MacRae[1], C Farquharson[1]
[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, UK

MEPE is mainly expressed in mineralising tissues, and has been implicated in osteoblast matrix mineralisation directly by releasing an ASARM peptide. Although the growth plates of MEPE transgenic mice display severe morphological disruption, the expression and function of MEPE in growth plate mineralisation remains largely undefined.

Proximal tibiae from 3-week-old wild-type mice were analysed for Mepe expression by in situ hybridisation and growth plate microdissection. Gene expression by the ATDC5 chondrogenic cell line was examined by RT-qPCR over a 20-day culture period under calcifying conditions. 20μM phosphorylated (pASARM) and non-phosphorylated ASARM (npASARM) peptides were added to ATDC5 cultures, and to 17-day-old and 15-day-old embryonic metatarsal explants.

Mepe expression was abundant throughout the growth plate as shown by in situ hybridisation. Microdissection of the growth plate confirmed an increased expression of Mepe in the hypertrophic chondrocytes (9-fold increase compared to proliferative chondrocytes, $P<0.05$). ATDC5 cells showed an initial decrease in Mepe expression at day-10 of culture, after which expression increased at day-15. Treatment of ATDC5 cells with pASARM peptide caused an inhibition of mineralisation, determined by alizarin red staining ($P<0.01$). Treatment with npASARM promoted mineralisation ($P<0.01$). The growth rate of 15 and 17-day-old embryonic metatarsals and the proliferation of chondrocytes within were not affected by treatment with 20μM pASARM or npASARM peptides. This indicates that the peptides were not toxic and the bones were still viable after 7 days of culture. However, in 17-day-old embryonic metatarsals, the growth of the central diaphyseal mineralisation zone was inhibited in bones treated with 20μM pASARM ($P<0.001$). This was further examined in 15-day-old embryonic metatarsals in which the central mineralised core only forms after 7 days in culture. Treatment with 20μM pASARM peptides completely prevented mineralisation. Alkaline phosphatase activity was unchanged between the treated and untreated bones, suggesting that the observed inhibition of mineralisation is not a result of decreased enzyme activity.

Our findings indicate that MEPE is expressed by growth plate chondrocytes and that it is likely to have a developmental role in the inhibition of cartilage matrix mineralisation through its cleavage and subsequent phosphorylation of the ASARM peptide.

OC25

THE EFFECT OF RECOMBINANT HUMAN FIBROBLAST GROWTH FACTOR-18 ON ARTICULAR CARTILAGE FOLLOWING SINGLE IMPACT LOAD

LV Barr*[1], FMD Henson [1], A Getgood [1], N Rushton [1]
[1] Orthopaedic Research Unit, University of Cambridge, UK

Background: Mechanical trauma to articular cartilage is a known risk factor for Osteoarthritis (OA). The application of single impact load (SIL) to equine articular cartilage is described as a model of early OA changes and shown to induce a damage/repair response. Recombinant Human Fibroblast Growth Factor-18 (rhFGF-18) has been previously shown to have anabolic effects on chondrocytes in vitro. The aim of this in vitro study was to ascertain the effect of rhFGF-18 on the repair response of mechanically damaged articular cartilage.
Methods: Articular cartilage discs were harvested from healthy mature horses (n=4) and subjected to single impact load using a drop tower device. The impacted explants, together with unimpacted controls were cultured in modified DMEM +/- 200ng/ml rhFGF-18 for up to 30 days. Glycosaminoglycan (GAG) release into the media was measured using the dimethylmethylene blue (DMMB) assay, aggrecan neopepitope CS846 and Collagen Propeptide II (CPII) were measured by ELISA. Histological analysis, immunohistochemistry and TUNEL staining were used to assess proteoglycan content, type II and type VI collagen localisation, cell morphology, repair cell number and cell death.
Results: Impacted explants treated with rhFGF-18 showed significantly more GAG release and CS846 release into the media compared to other experimental groups ($p<0.05$), but no significant increase in CPII levels. Loaded sections treated with rhFGF-18 had increased type II and VI collagen immunohistochemistry scores, more repair cells on the tissue surface and significantly less cell death ($p<0.001$) compared to other experimental groups at day 30 in culture.
Conclusion: In an in vitro damage/repair model, rhFGF-18 increases the proteoglycan synthesis, collagen type II and VI protein within sections and the repair cell number and prevents apoptosis at Day 30. This suggests that rhFGF18 may be a good candidate for enhancement of cartilage repair following mechanical damage.

OC26

THE APPEARANCE AND MODULATION OF OSTEOCYTE MARKER EXPRESSION DURING CALCIFICATION OF VASCULAR SMOOTH MUSCLE CELLS

Zhu D*[1], Mackenzie NCW[1], Millán JL[2], Farquharson C[1], MacRae VE[1]
[1]The Roslin Institute, The University of Edinburgh, UK;[2]Sanford Children's Health Research Center, Sanford-Burnham Medical Research Institute, USA

Vascular calcification has severe clinical consequences in a number of diseases, including diabetes, atherosclerosis and end-stage renal disease. Vascular smooth muscle cells (VSMCs), the predominant cell type involved in medial vascular calcification, can undergo phenotypic transition to both osteoblastic and chondrocytic cells within a calcifying environment. In the present study, using in vitro VSMC calcification studies in conjunction with ex vivo analyses of a mouse model of medial calcification, we show that vascular calcification is also associated with the expression of osteocyte phenotype markers. As controls, the terminal differentiation of murine calvarial osteoblasts into osteocytes was induced in vitro in the presence of calcifying medium (containing bgp and ascorbic acid), as determined by increased expression of the osteocyte markers DMP-1, E11 and sclerostin. Culture of murine aortic VSMCs under identical conditions confirmed that the calcification of these cells can also be induced in similar calcifying medium. Calcified VSMCs had increased alkaline phosphatase activity and PiT-1 expression, which are recognized markers of vascular calcification. Expression of DMP-1, E11 and sclerostin was up-regulated during VSMC calcification in vitro. Increased protein expression of E11, an early osteocyte marker, and sclerostin, expressed by more mature osteocytes was also observed in the calcified media of Enpp1-/- mouse aortic tissue. This study has demonstrated the up-regulation of key osteocytic specific molecules during the vascular calcification process. A fuller understanding of the functional role of osteocyte formation and specifically sclerostin and E11 expression in the vascular calcification process may identify novel potential therapeutic strategies for clinical intervention.

OC27

DOES A STRONTIUM SUBSTITUTED BIOGLASS ENHANCE THE RATE OF BONE INGROWTH INTO A CRITICAL SIZE DEFECT COMPARED TO A COMMERCIALLY AVAILABLE TCP-CASO4?

+; Allen F*[1]; Blunn G[1]; McCarthy ID[1]; O'Donnell M[2]; Stevens MM[3]; Goodship AE[1];

+1UCL Institute of Orthopaedics, London, UK, 2RepRegen Ltd, London, UK, 3Imperial College London, London, UK

Synthetic bone grafts are used in several major dental and orthopaedic procedures. Strontium, in the form of strontium ranelate, has been shown to reduce fracture risk when used to treat osteoporosis. The aim of the study was to compare bone repair in femoral condyle defects filled with either a 10% strontium substituted bioactive glass (StronBoneTM) or a TCP-CaSO4 graft. We hypothesise that strontium substituted bioactive glass increases the rate of bone ingrowth into a bone defect when compared to a TCP-CaSO4 ceramic graft.
A critical size defect was created in the medial femoral condyle of 24 sheep; half were treated with a Sr-bioactive glass (StronBoneTM), and in the other animals defects were filled TCP-CaSO4. Two time points of 90 and 180 days were selected. The samples were examined with regard to: bone mineral density (BMD) from peripheral quantitative CT (pQCT), mechanical properties through indentation testing, and bony ingrowth and graft resorption through histomorphometry.
The radiological density of Sr-bioactive glass in the defect is significantly higher than that of the TCP-CaSO4-filled defect at 90 and 180 days, ($p=0.035$ and $p=0.000$). At 90 days, the stiffness of the defect containing Sr-bioactive glass and is higher than that of the TCP-CaSO4 filled defect, ($p=0.023$). At 6 months there is no significant difference between the two materials. Histomorphometry showed no significant difference in bone ingrowth at any time point, however significantly more of the graft is retained for the StronBoneTM treatment group than the TCP-CaSO4 group at both 0 days ($p=0.004$) and 180 days ($p=0.000$). The amount of soft tissue within the defect was significantly less in the StronBoneTM group than for the TCP-CaSO4 group at 90 days ($p=0.006$) and 180 days ($p=0.000$)
The data shows the mechanical stability of the defect site is regained at a faster rate with the strontium substituted bioglass than the TCP-CaSO4 alternative. Histomorphmetry shows this is not due to increased bone ingrowth but may be due to the incorporation of stiff graft particles into the trabeculae. Sr-bioactive glass produces a stronger repair of a femoral condyle defect at 3 months compared with TCP-CaSO4.

OC28

INTRA-ARTICULAR AMPA/KAINATE GLUTAMATE RECEPTOR ANTAGONISTS ALLEVIATE INFLAMMATION AND PAIN IN RAT ANTIGEN INDUCED ARTHRITIS

CS Bonnet*[1], AS Williams[2], DJ Mason[1]
[1]Pathophysiology and Repair Division, School of Biosciences, Cardiff University, Museum Avenue, Cardiff, CF10 3AX; [2]Rheumatology Research Laboratory, Cardiff University, Heath Park, Cardiff, CF14 4XN

Concentrations of the neurotransmitter glutamate are greatly increased in synovial fluids of RA and OA patients, where they correlate with cytokine concentrations. Previously, we demonstrated that human synoviocytes express functional glutamate receptors (GluRs) and that activation of NMDA GluRs decreases proMMP2 expression, whilst activation of kainate GluRs increases IL-6 release (1). High glutamate concentrations in the joint cause arthritic pain which is alleviated by intra-articular injection of GluR antagonists. However, the effects of such inhibitors on arthritis progression via activation of GluRs expressed on other joint tissues have not been considered. We are investigating the hypothesis that specific GluR subunits in the arthritic synovium mediate proinflammatory, degradative and proliferative responses, and may be therapeutically targeted to reduce disease progression and pain.
Using the mono-articular antigen induced arthritis (AIA) rat model, we used intra-articular injection of NBQX to inhibit AMPA/kainate receptors at the time of arthritis induction, prior to peak IL-6 levels. Over a 21 day period, we measured knee swelling and gait patterns from AIA, AIA + NBQX and normal rats (n=6 for each group). A combination of motion analysis, using Qualisys Pro-Reflex MCU-1000 cameras, and footprint characteristics revealed limping and abnormal movements as an indirect measure of pain. On day 21, joint tissues were taken for reverse transcription PCR assays, immunohistochemistry and histology to examine GluR expression and joint destruction.
Significantly less knee swelling ($P<0.0006$) was found in NBQX treated rats compared to AIA rats. Using a footprint severity scoring system on a scale of 0 (entire footprint) to 4 (no footprint at all), NBQX treated rats displayed significantly less pain related behaviour during the initial flare of arthritis compared to AIA rats ($P=0.0002$). Ionotropic and metabotropic GluR mRNA was differentially expressed in cartilage, synovium, meniscus, fat pad, patella, femoral head and shaft of rat knees.
We have shown that intra-articular NBQX treatment alleviates inflammation and pain in arthritis in vivo and that GluRs are differentially expressed in knee joint tissues. This supports our hypothesis that kainate GluRs may be specifically targeted to ease pain, inflammation and pathology in arthritis.
(1) Flood et al. (2007) Arthritis Rheum, 56: 2523-2534.

OC29

AN INVESTIGATION OF RELATIONSHIPS BETWEEN FRACTURE RISK, TOTAL BODY DXA AND TIBIAL pQCT REVEALS INDEPENDENT INFLUENCES OF AREAL AND CORTICAL BMD: IS THERE A CASE FOR COMBINING DXA AND pQCT IN ASSESSING FRACTURE RISK?

Adrian Sayers*[1], Jacky Chau[1], Kate Tilling[2] and Jon Tobias[1]
Musculoskeletal Research Unit, University of Bristol, UK; [2] School of Social and Community Based Medicine, University of Bristol, UK

Introduction: Areal bone mineral density (aBMD) as measured by total body DXA has previously been found to be related to fracture risk in children, particularly when adjusted for body size, possibly reflecting an influence of true volumetric bone density. Likely constituents of volumetric bone density as measured by pQCT, such as cortical thickness and cortical BMD, are also related to fracture risk. However, to what extent DXA and pQCT evaluate equivalent determinants of fracture risk is unknown. To address this question we examined whether, in the Avon Longitudinal Study of Parents and Children, area and cortical BMD, measured by total body DXA and pQCT respectively, are independently related with fracture risk.

Methods: Total body DXA and mid-tibial pQCT were linked to fracture history in the preceding three years in 2849 boys and girls mean 15.5 years of age. Fracture risk was investigated using multivariable logistic regression. Relative risk and absolute risk differences were calculated for minimally adjusted (age) and more fully adjusted models (height, fat and lean mass).
Results: In adjusted models aBMD and cortical BMD were both inversely associated with fracture risk: 25.3% reduction in odds of fracture per SD increase in aBMD (b=0.747 [95%CI: 0.636, 0.878], p=0.0004, r2=0.047); 24.1% reduction in odds of fracture per SD increase in cortical BMD (b=0.759 [95%CI: 0.660, 0.874], p=0.0001, r2=0.048). In a combined model, although individual associations were marginally attenuated, SD increases in aBMD and cortical BMD were still associated with 19.8% and 20% reductions in odds of fracture respectively (baBMD=0.802, [95%CI: 0.678, 0.949], p=0.010/ bvBMD=0.8 [95%CI: 0.691, 0.926],p=0.0029, r2=0.051). Finally, the absolute risk reduction between lower and upper quartiles of aBMD (-4.5%) and cortical BMD (-4.3%), was significantly improved when both were used in combination (-6.6%) p<0.001.
Conclusions: Whereas fracture risk in adolescence is related to both aBMD and cortical BMD, the inclusion of cortical BMD into a DXA based model of risk significantly improves fracture risk prediction, implying that these factors act in part independently. Further studies are justified to examine the utility of combined use of DXA and pQCT to evaluate fracture risk not only in adolescents, but also in older populations.

OC30
IRON STATUS, FGF23 AND RICKETS IN THE GAMBIA
V Braithwaite*[1], LMA Jarjou[2], GR Goldberg[1,2], A Prentice [1,2]
[1] MRC Human Nutrition Research, Cambridge, UK;[2] MRC Keneba, The Gambia
Recent studies have suggested a relationship between iron status and circulating fibroblast growth factor-23 (FGF23). Serum ferritin has been identified as a negative predictor of FGF23 [1] and conversely FGF23 has been shown to be elevated by intravenous injections with iron polymaltose [2].
Iron deficiency anaemia is common in The Gambia and elevated FGF23 has been implicated in the aetiology of Gambian rickets [3]. This study aimed to assess the prevalence of elevated FGF23 and low haemoglobin (Hb) in Gambian children (0.5-18yrs) and to investigate whether a relationship exists between them.
We conducted a retrospective analysis on data from children with a personal or family history of rickets-like bone deformity (BD, n=115). Data from local community children (LC, n=369) were used for comparison. Hb was analysed in fresh, overnight-fasted blood samples on a blood-gas analyser (ABL77) and WHO definitions for anaemia were used. C-terminal FGF23 was measured by Immutopics; the upper-limit reference range was 150 RU/ml. Linear regression was used to determine the relationship between Hb and FGF23 (both transformed to natural logarithms) with adjustments for age and sex. Logistical models were used for categorical analysis.
14% of BD were anaemic compared to 6% of LC (p=0.001). 10% of BD had FGF23>900 RU/ml compared to 1% of LC (p<0.0001). There was a significant correlation between FGF23 and Hb (lnFGF23=9.46-1.73lnHb, R2=16.0%, p<0.0001). When split between groups, Hb was a strong negative predictor of FGF23 in BD (lnFGF23=17.71-4.96lnHb, R2=27.2%, p<0.0001) but only weakly for LC (lnFGF23=6.98-0.82lnHb, R2=9.9%, p=0.03). The Hb x group interaction was highly significant (p<0.0001) demonstrating differences between BD and LC in the slopes of the relationship.
The findings that Hb is a negative predictor of FGF23, that the slope is steeper in BD and that high FGF23 and low Hb are more prevalent in BD suggest that iron maybe involved in FGF23 metabolic pathways and that there is a link between Hb and FGF23 in the aetiology of Gambian rickets.
1. Durham, B.H., et al. Assoc Clin Biochem 2007.44:p.463-466.
2. Schouten, B.J., et al. J Clin Endocrinol Metab 2009.94:p.2332-2337.
3. Prentice, A., et al. Bone 2008.42:p.788-797.

OC31
TESTING THE MECHANOSTAT THEORY: THE RELATIONSHIP BETWEEN CT BONE AND MUSCLE VARIABLES IN MID-THIGH IN ELDERLY MEN AND WOMEN AND ASSOCIATION WITH INCIDENT LOWER LIMB FRACTURES
F Johannesdottir*[1], T Aspelund[1,2], K Siggeirsdottir[2], BY Jonsson[3], B Mogensen[1,4], S Sigurdsson[2], TB Harris[5], TF Lang[6], V Gudnason[1,2], G Sigurdsson[1,2,4]
[1]University of Iceland, Reykjavik, Iceland; [2]Icelandic Heart Association, Kopavogur, Iceland; [3]University Hospital, Malmö, Sweden; [4]Landspitali-University Hospital, Reykjavik, Iceland; [5]National Institute on Aging, Bethesda, MD, USA; [6]University of California, San-Francisco, CA, USA
Background: The mechanostat theory suggests a fixed ratio between bone and muscle as an adaptation to mechanical loading explaining the loss of bone and increasing fracture risk with age by sarcopenia.
Methods and study group: In this cross-sectional study we investigated the relationship between muscle and bone parameters in mid-thigh in elderly men and women using computed tomography (CT). Additionally we have studied the association of these variables with incident low trauma lower limb fractures. A total of 3546 elderly individuals (1786 women, 1760 men) age 67-93 years, participants in a population-based study, the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavík) were studied and of those 109 women and 64 men sustained a fracture during follow-up of 4.2 years. We used a single axial CT section through the mid-thigh (cross-sectional muscular area, medullary area, cortical area and thickness, buckling ratio, the ratio of bone radius to cortical thickness). Associations between variables were estimated by Pearson's correlations. The predictive power of these measures for fractures was tested by Cox proportional hazards regression.
Results: The muscular area declined with 10 years in age similarly in both sexes. The increment in medullary area in mid femur with age was fourfold greater in women than in men. The cortical thickness declined significantly in both sexes but twofold more in women. Cortical area was positively associated with muscular area in both sexes and remained significant after adjustment for body size and age (r=0.16, p<0.001), explaining less than 5% of variability in cortical area. The association between muscular area and medullary area was minimally significant in women but not in men after correcting for body size and age. Small muscular area, large medullary area and high buckling ratio were significant predictors of fractures after adjustment for age and BMI in both sexes. The muscular area conferred significant protection against fracture independently of buckling ratio and meduallary area.
Conclusion: The findings suggest that there are only weak correlations between muscles in mid-thigh and bone parameters which predict lower limb fractures after adjusting for body size. However, muscle parameters seem to be protective against fractures indirectly, independent of bone parameters.

OC32
CIRCULATING FIBROBLAST GROWTH FACTOR-23 (FGF-23) FOLLOWING VITAMIN D SUPPLEMENTATION IN VITAMIN D INSUFFICIENCY
R Inaoui*[1], WD Fraser[2], I Fogelman[1],G Hampson [1]
[1]Osteoporosis Clinic, Rheumatology, Guy's Hospital, London UK;[2]Department of Clinical Chemistry, Royal Liverpool Hospital, Liverpool, UK
1,25 dihydroxy vitamin D up-regulates Fibroblast Growth Factor -23 (FGF-23). Both are implicated in a feedback loop, as increases in FGF-23 inhibit 1, 25 dihydroxy vitamin D synthesis. High dose vitamin D supplements are frequently given to patients with low 25(OH) vitaminD who are at high fracture risk. We investigated the effect of high dose vitamin D on circulating FGF-23 concentrations and the interaction between FGF-23 and 1, 25 dihydroxy vitaminD in patients with vitamin D deficiency /insufficiency.
Thirty one subjects (11M aged (mean [SD]) 56.6 [19.3] and 20 F aged 62.7 [15.3] years were studied. They were administered an intra-muscular injection of 300,000 I.U of Ergocalciferol (D2) and prescribed daily supplements of calcium (1.2 g) and 800 I.U colecalciferol (D3). Blood samples were obtained at baseline, 1, 2 and 3 months for measurement of serum calcium, phosphate, PTH, 25 (OH) vitamin D and FGF-23. 1,25dihydroxyvitamin D was measured at baseline and 3 months.
Serum 25 (OH) vitamin D increased significantly (baseline mean [SD] 33.5 [14.9], 50.5 [21.6] nmol/L at 3 months *($p < 0.05$)*. PTH decreased from baseline, but not significantly (baseline: 64[60], 3 months: 50[28.5] ng/L). There was no significant difference in serum phosphate. FGF-23 concentrations increased significantly (baseline: 85.3[44.1], 1 month : 111[58], 2 month : 99.6 [62], 3 month: 108[59] RU/ml *($p <0.05$)*. A positive correlation was seen between FGF-23 and serum phosphate at 3 months *($r= 0.44$, $p= 0.02$)*. 1, 25 dihydroxyvitamin D increased in 18 patients (Group A : baseline 107 [39] , 3 month 134[34.5] pmol/L) and decreased in 13 patients (Group B : baseline 158.6 [40], 3 months 123 [37] pmol/L *($p<0.05$)*. Baseline 1, 25 dihydroxy-vitamin D was significantly higher in Group B compared to Group A *($p<0.001$)*. There was no significant difference in PTH. A larger increase in FGF-23 was seen in Group B (Group B mean [SEM] % change from baseline; 1 month: 90[47], 2 month: 84[44], 3 month: 45[16], Group A; 1 month: 36[12.5], 2 month: 14 [10], 3 month: 29 [15] *($p=0.039$)*. These data suggest that FGF-23 is a counter-regulatory hormone for 1, 25 dihydroxy vitamin D production.

OC33
OBESITY AND FRACTURES IN POSTMENOPAUSAL WOMEN: THE GLOBAL STUD YOF OSTEOPOROSIS IN WOMEN (GLOW)
JE Compston*[1], NB Watts[2], R Chapurlat[3], C Cooper[4], S Boonen[5], S Greenspan[6], J Pfeilschifter[7], S Silverman[8], A Diez-Pérez[9], R Lindsay[10], KG Saag[11], JC Netelenbos[12], S Gelbach[13], FH Hooven[13], J Flahive[13], JD Adachi[14], M Rossini[15], AZ LaCroix[16], C Roux[17], PN Sambrook[18], ES Siris[19]
[1]Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; [2]Bone Health and Osteoporosis Center, University of Cincinnati, Cincinnati, Ohio, USA;[3]INSERM Research Unit 831, Université de Lyon, Department of Orthopedics and Rheumatology, Hôpital E Herriot, Lyon, France;[4]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton General Hospital, and Norman Collisson Chair of Musculoskeletal Sciences, University of Oxford, Oxford, UK;[5]Leuven University Center for Metabolic Bone Diseases and Division of Geriatric Medicine, Katholieke Universiteit Leuven, Leuven, Belgium;[6]University of Pittsburgh, Pittsburgh, Pennsylvania,

USA;[7]Alfried Krupp Krankenhaus, Department of Internal Medicine III, Essen, Germany;[8]Cedars-Sinai Medical Center, Los Angeles, California, USA;[9]Hospital del Mar-IMIM-Autonomous University of Barcelona, and RETICEF, Instituto Carlos III, Barcelona,Spain;[10]Columbia University, and Regional Bone Center, Helen Hayes Hospital, West Haverstraw, New York, USA;[11]University of Alabama-Birmingham, Division of Clinical Immunology and Rheumatology, Birmingham, Alabama, USA;[12]Department of Endocrinology, VU University Medical Center, Amsterdam, The Netherlands;[13]Center for Outcomes Research, University of Massachusetts Medical School, Worcester, Massachusetts, USA;[14]St Joseph's Healthcare, McMaster University, Hamilton, Ontario, Canada;[15]Department of Rheumatology, University of Verona, Ospedale, Verona, Valeggio, Italy;[16]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA;[17]Paris Descartes University, Cochin Hospital, Paris, France;[18]University of Sydney-Royal North Shore Hospital, St. Leonards, Sydney, New South Wales, Australia;[19]Columbia University Medical Center, New York, USA

Low body mass is an important risk factor for fracture whilst obesity is widely believed to be protective. However, we recently reported a high prevalence of obesity in postmenopausal women with fragility fracture presenting to our Fracture Liaison Service. The aim of this study was to examine the prevalence and incidence of clinical fractures in obese postmenopausal women recruited in the Global Osteoporosis study in Women (GLOW}, a multinational, prospective, population-based observational study in 60,393 women. Data were collected using self-administered questionnaires covered domains that included patient characteristics, fracture history, risk factors for fracture, and anti-osteoporosis therapy

Fracture history and body mass index (BMI) were available at baseline and 2 years in 44,534 women, 23.4% of whom were obese (BMI 30 kg/m2 or greater). In obese women, the prevalence of fractures at baseline was 222 per 1,000 and incidence at 2 years was 61.7 per 1,000, similar to rates in non-obese women (227 and 66.0 per 1,000, respectively). Fractures in obese women accounted for 23% and 22% of all previous and incident fractures, respectively. Higher BMI was associated with ankle and lower leg fractures and inversely related to wrist and pelvis fractures. Obese women with fracture were more likely to have experienced early menopause and to report two or more falls in the past year (P <0.001). Self-reported asthma, osteoarthritis, and type 1 diabetes were all significantly associated with higher BMI (P <0.001). At 2 years, only 27% of obese women with incident fracture were receiving bone-protective medication, compared with 41% of non-obese and 57% of underweight women.

Our results demonstrate that nearly one in four postmenopausal women with a clinical fracture is obese and that obesity is a risk factor for fractures of the lower leg and ankle. Our findings have major public health implications in view of the rapidly rising incidence of obesity. Further studies are required to establish the pathogenesis of fractures in the obese population and to develop effective strategies for their prevention.

OC34

RELATIONSHIPS BETWEEN FAT MASS, PLASMA ADIPOKINES AND BONE IN POST-MENOPAUSAL CAUCASIAN WOMEN

KA Ward*, REB Webb, A Prentice, GR Goldberg

MRC Human Nutrition Research, Cambridge, UK

Until recently, obesity was assumed to protect against fragility fracture and was attributed to weight-bearing effects of excess adipose tissue. However, adipokines, secretory products of adipose tissue, may have independent adverse or beneficial effects on bone. The high prevalence of obesity in many populations, together with recent findings in the UK of a high prevalence of obese fragility fracture patients with normal BMD, and increased fracture risk in obese children, indicate that studies are needed to establish bone phenotype in obesity.

We investigated relationships between adipokines and bone independently of body weight and composition in a group of 70 post-menopausal women of a wide range of BMI and adiposity: mean±SD (range) age 62.3±3.7 y (55.5-70.9); weight 67.5±11.3 kg (49.2-100.0); BMI 24.9±3.8 kg/m2 (17.6-33.3). Whole-body, hip, spine and forearm DXA scans were taken. Size-adjusted BMC (SA-BMC) was generated by adjusting for bone area, weight and height. Total body fat mass (TBFM) was measured using a 4-compartment model (total-body water [deuterium dilution], total BMC [DXA], body volume [air-displacement plethysmography], body weight). Fasting plasma concentrations of leptin, total and high-molecular weight (HMW) adiponectin were measured.

Relationships were examined using univariate and multiple regression analyses. All measures were transformed into natural logarithms. Total adiponectin was retained in regression models regardless of significance.

Mean TBFM was 26.5±8.9 kg (11.6-49.2). There were significant associations between TBFM and leptin (0.838), TBFM and adiponectin (-0.333), adiponectin and weight (-0.402), and adiponectin and leptin (-0.433). Fat mass was a predictor of radius (P=0.0008) and forearm (P=0.04) SA-BMC. Leptin was a predictor of radius SA-BMC (0.05). SA-BMC at several forearm sites (P=0.02-0.003), trochanter (P=0.01), hip shaft (P=0.04) and whole-body (P=0.05) was negatively associated with total adiponectin. SA-BMC of the forearm (p=0.01) and trochanter (p=0.03) was negatively related to HMW adiponectin.

In contrast to leptin, adiponectin was negatively associated with bone measures irrespective of whether TBFM was also associated with the same measure. These findings suggest that relationships between adiponectin and bone are more than simply those of a marker of TBFM. Adiponectin may partly explain increased fractures in obese individuals where hip fractures are more common than non-obese.

OC35

THE HIGH BONE MASS PHENOTYPE IS CHARACTERISED BY INCREASED SUBCUTANEOUS AND INTRA-MUSCULAR FAT, BUT DECREASED MARROW FAT

CL Gregson*[1], A Sayers[1], SA Steel[2], V Lazar[2], J Rittweger[3,4], JH Tobias[1]

[1]Musculoskeletal Research Unit, School of Clinical Sciences, University of Bristol, Bristol, UK;[2]Hull Royal Infirmary, Hull, UK;[3]Bone Metabolism Unit, Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University, Manchester, UK; [4]Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany

In our unique case-control study of High Bone Mass (HBM), cases were found to have greater total body fat mass and lower platelet levels compared to controls. To explore inter-relationships between fat, bone and bone marrow suggested by these results, we aimed to characterise adipose tissue distribution in HBM individuals using a novel application of tibial pQCT.

196 HBM index cases were identified by screening a hospital DXA database (n=105,333). HBM was defined as a) L1 Z-score of 3.2 or more and total hip Z-score of 1.2 or more, or b) total hip Z-score 3.2 or more. Cases with significant osteoarthritis and/or other causes of raised BMD were excluded. Relatives and spouses were recruited, in whom HBM affection status was defined as L1 Z plus total hip Z-scores of 3.2 or more. Controls comprised unaffected relatives and spouses. pQCT was performed at the tibial diaphysis (66% of its length), using a Stratec XCT2000L. Cases were compared with controls using linear regression, adjusting for age, gender, height and menopausal status.

98 HBM cases (71 index, 27 affected relatives) & 65 controls (43 unaffected relatives, 22 spouses) underwent pQCT; 81.6% & 50.8% were female, mean age 58 & 55 years, height 167 & 171 cm, weight 86 & 82 kg respectively; all Caucasian. As shown in the table, compared with controls, HBM cases had 22% more intra-muscular fat and considerably greater calf muscle and subcutaneous fat areas; however, marrow cavity area was reduced at the expense of marrow fat rather than haemopoietic tissue. Interestingly, after further adjustment for body weight, HBM cases still had greater intra-muscular fat than controls (mean difference [95% CI], 1.79 [0.22, 3.37]cm2, p=0.025) and marrow results remained unchanged.

In summary, HBM individuals have greater subcutaneous and intra-muscular fat tissue, whilst in contrast marrow fat is reduced, possibly reflecting a mechanism to maintain haemopoietic function within the reduced marrow cavity of HBM long bones. Whether increased intra-muscular fat in HBM cases has a detrimental effect upon muscle function, so explaining the reduction in exercise tolerance previously observed amongst HBM cases, remains to be determined.

OC36

ENHANCED WEAR AND CORROSION IN MODULAR TAPERS IN TOTAL HIP REPLACEMENT - AN IN-VITRO BIOMECHANICAL STUDY

J. Meswania*, G. Biring, C.Wylie, J.Hua S. Muirhead-Allwood, G. Blunn

John Scales Centre for Biomedical Engineering, Institute of Orthopaedics and Musculo-Skeletal Science, University College London, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore,Middlesex, HA7 4LP

Introduction The National Joint Registry has recently identified failure of large head metal on metal hip replacements. This failure is associated with the high torque at the interface of standard modular taper junction leading to fretting and corrosion. A number of manufacturers produce mini spigots, which in theory, provide a greater range of motion as the neck head junction is reduced. However, the relative torque to interface ratio at this junction is also increased. In this study we investigated hypothesis that the use of small spigots (minispigots) will increase wear and corrosion on modular tapers.

Methods Wear and corrosion of spigots were compared in-vitro when loaded with a force representative of the resultant force passing through the hip. The heads (female tapers) were made of cobalt-chrome-molybdenum (CoCrMo) and the stems (male tapers) of titanium alloy (Ti). Commercially available tapers and heads were used. The surface parameters & profiles were measured before & after testing. Electrochemical static and dynamic corrosion (pitting) tests were performed on minispigots under loaded and non-loaded conditions.

Results Post-testing the surface parameters Ra, Ry & Rz on the head taper associated with the minispigots had become greater compared with standard spigots. In all instances the profile of the titanium male tapers was unchanged. SEM showed the corroded region of the head was similar to the profile on the Ti male taper, with evidence of pitting in the cobalt chrome. In the CoCrMo/ Ti combinations, wear and corrosion were increased in minispigots compared with standard spigots. On minispigots the rough surface finishes were affected more severely than those with a smoother surface. Static corrosion tests showed evidence of fretting in the rough but not the smooth minispigots. Pitting scans showed a greater hysteresis with the rough surface finishes on the minispogot indicating potentially greater corrosion in the former.

Conclusion The relative size of the taper in comparison to the head combined with the surface finish was crucial. As the relative torque to interface ratio at

this junction increased corrosion of the cobalt chrome head increases and is further enhanced if the surface finish on the tapers is rough.

OC37

MEASUREMENT OF SKIN CAPACITANCE: A NOVEL METHOD OF DIAGNOSING AUTONOMIC DYSFUNCTION IN CARPAL TUNNEL SYNDROME

SK Dheerendra* [1], WS Khan [2], P Smitham [3], NJ Goddard [4]

[1] Department of Trauma & Orthopaedics, Macclesfield District General Hospital, UK; [2] Royal National Orthopaedic Hospital, Stanmore, UK; [3] Department of Trauma & Orthopaedics, Royal Free Hospital, Pond Street, London

Background & Objectives: Sensory and motor manifestations in carpal tunnel syndrome (CTS) are well documented, whereas the associated autonomic dysfunction is often overlooked. The aim of this study is to demonstrate that autonomic dysfunction of the CTS hands can be quantified by measuring skin capacitance.

Methods: Patients with clinical and electrophysiological signs of idiopathic carpal tunnel syndrome meeting the inclusion criteria were recruited. The patients were also scored based on the Brigham carpal tunnel severity score. Skin capacitance was measured using Corneometer CM825 (C&K Electronic, GmbH). The measurements were taken from the palmar aspect of distal phalanx of the index and little finger of the affected hand. Normal healthy patients with no signs and symptoms of carpal tunnel syndrome were recruited as controls and skin capacitance was measured in a similar fashion as the CTS group.

Results: The CTS group consisted of 25 patients (18 female & 7 male) and 35 hands with an average age of 59.2 years (33-83 years). The mean symptom severity score was 2.80 (1.27-4.18; SD 0.82) and functional status score was 2.53 (1-4.26; SD 1.08). The mean ratio of skin hydration between the index and little finger was 0.85 (0.6-1.25; SD 0.155). Using the paired t-test to determine paired differences between index and little finger measurements, the mean difference was 12.6 ($p<0.001$).

The control group consisted of 15 people (9 female and 6 male) and 30 hands. The average age was 47.3 years. The mean ratio of skin hydration between the index and little finger was 0.97 (0.77-1.42 SD 0.105). Using the paired t-test to determine paired differences between index and little finger measurements, the mean difference was 1.31 ($p=0.317$). The difference in skin hydration between the index and little finger was directly compared between the controls and CTS group, this difference was statistically significant, $p=0.002$.

Conclusion: A simple method to determine dysautonomia, using Corneometer CM825, by the clinician has been demonstrated. Measurement of skin capacitance will reduce the dependence on electrophysiological studies, thus reducing the time for arriving at a diagnosis, improved patient satisfaction and cost-effectiveness.

OC38

T-2 TOXIN DISRUPTS THE EXPRESSION OF CHONDROITIN SULPHATED SULPHATION MOTIFS IN ARTICULAR CARTILAGE FROM AN ANIMAL MODEL OF KASHIN-BECK DISEASE

S Li*[1], J Chen[2], B Caterson[1], CE Hughes[1]

[1]Connective Tissue Biology Laboratories, School of Biosciences, Cardiff University, UK;[2]The Endemic Disease Institute, Medical School, Xi'an Jiaotong University, Xi'an, China

Introduction: Kashin-Beck disease (KBD) is an endemic degenerative osteoarthropathy affecting approximately 3 million people in China (Stone R, 2009). The precise aetiology of KBD is not clear, but the lack of selenium and the pollution of mycotoxins in food are a suspected cause of KBD. In this pilot study, we use a rat model to investigate the effect of low selenium and T-2 toxin on articular cartilage metabolism.

Methods: 140 male Sprague-Dawley rats were fed with selenium-deficient or normal diet for 4 weeks to produce a low selenium or normal nutrition status. The rats were then fed for a further 4 weeks with low selenium or normal diets with or without T-2 toxin (100ng per gram body weight per day). The rat knee joints were fixed and paraffin embedded and histological and immunohistochemical staining was performed to analyse the metabolism of articular cartilage.

Results: There was increased cell cluster formation in the middle and/or deep zones in rats fed with both diets. However, an apparent cell loss was observed in the low selenium + T-2 toxin group with an apparent increase in caspase-3 staining, indicating the increased cell apoptosis. Moreover, toluidine blue staining was reduced in the low selenium + T-2 toxin group, suggesting a loss of sulphated glycosaminoglycans. Similarly, there was reduced 2B6 and 6C3 staining in the territorial matrix of chondrocytes, indicating a reduced synthesis in 4-sulhated and native CS motifs. In contrast, increased 1B5 staining was observed in the articular cartilage from the low selenium + T-2 toxin group, suggesting a lack of CS sulphatransferase activity. Interestingly, there was increased 7D4 staining in the superficial zone of articular cartilage from low selenium + T-2 toxin group, suggesting an initiation of an osteoarthritis-like lesion.

Discussion: These results indicated that low selenium nutrition and T-2 toxin could promote cell apoptosis and disrupt CS-GAG metabolism in ECM of rat articular cartilage in this animal model, which is similar to that observed in KBD patients. Collectively, our results support the hypothesis that low selenium and T-2 toxin are the cause of KBD.

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OC39

SPATIAL AND TEMPORAL STUDY OF BONE MARROW LESIONS IN OSTEOARTHRITIS, AND THEIR RELATIONSHIP TO DENUDED ARTICULAR CARTILAGE

SWD McLure*[1], MA Bowes[2], CBH Wolstenholme[2],GR Vincent[1], S Williams[1], R Maciewicz[3], JC Waterton [3], AJ Holmes [3], P Conaghan[1]

[1]University of Leeds, Leeds, UK; [2]Imorphics, Manchester, UK; [3]AstraZeneca, Macclesfield, UK

Bone marrow lesions (BMLs) have been extensively linked to the osteoarthritis (OA) disease pathway in the knee. Semi-quantitative evaluation has been unable to effectively study the spatial and temporal distribution of BMLs and consequently little is understood about their natural history. This study used a novel statistical model to precisely locate the BMLs within the subchondral bone and compare BML distribution with the distribution of denuded cartilage.

MR images from individuals (n=88) with radiographic evidence of OA were selected from the Osteoarthritis Initiative. Slice-by-slice, subvoxel delineation of the lesions was performed across the paired images using the criteria laid out by Roemer (2009). A statistical bone model was fitted to each image across the cohort, creating a dense set of anatomically corresponded points which allowed BML depth, position and volume to be calculated. The association between BML and denudation was also measured semi-quantitatively by visually scoring the lesions as either overlapping or adjacent to denuded AC, or not.

At baseline 75 subjects had BMLs present in at least one compartment. Of the 188 compartments with BMLs 46% demonstrated change greater than 727mm cubed, the calculated smallest detectable difference. The majority of lesions were found in medial compartments compared to lateral compartments and the patella (Figure 1A). Furthermore, in the baseline images 76.9% of all BMLs either overlapped or were adjacent to denuded bone. The closeness of this relationship in four individuals is shown in Figure 1B.

The distribution of lesions follows a clear trend with the majority found in the patellofemoral joint, medial femoro-tibial joint and medial tibial compartment. Moreover the novel method of measurement and display of BMLs demonstrates that there is a striking similarity between the spatial distribution of BMLs and denuded cartilage in subjects with OA. This co-location infers the lesions have a mechanical origin much like the lesions that occur in healthy patients as a direct result of trauma. It is therefore suggested that OA associated BMLs are in fact no different from the BMLs caused by mechanical damage, but occur as a result of localised disruption to the joint mechanics, a common feature of OA.

OC40

THE OPTIMISATION OF POLYMER TYPE AND CHAIN LENGTH FOR USE AS A BIOLOGICAL COMPOSITE GRAFT IN IMPACTION BONE GRAFTING: A MECHANICAL AND BIO-COMPATIBILITY ANALYSIS

ER Tayton*[1], S Fahmy[2], A Aarvold[1], JO Smith[1], S Kalra[1], A Briscoe[1],M Purcell[2], KM Shakesheff[2], S Howdle[2], DG Dunlop[1], ROC Oreffo[1].

[1]Bone and Joint Research Group, University of Southampton, Southampton, UK;[2]School of Chemistry, University of Nottingham, Nottingham,UK

Impaction bone grafting with milled human allograft is the gold standard for replacing lost bone stock during revision hip surgery. Problems surrounding the use of allograft include cost, availability, disease transmission and stem subsidence (usually due to shear failure of the surrounding allograft).

The aim of this study was to investigate various polymers for use as substitute allograft. The ideal graft would be a composite with similar mechanical characteristics as allograft, and with the ability to form de novo bone.

High and low molecular weight (MW) forms of three different polymers (polylactic acid (PLA), poly (lactic co-glycolic) acid (PLGA) and polycaprolactone (PCL)) were milled, impacted into discs, and then tested in a custom built shear testing rig, and compared to allograft.

A second stage of the experiment involved the addition of skeletal stem cells (SSC) to each of the milled polymers, impaction, 8 days incubation, and then tests for cell viability and number, via fluorostaining and biochemical (WST-1) assays.

The shear strengths of both high/ low MW PLA, and high/low MW PLGA were significantly higher than those of milled allograft ($P<0.001$, $P<0.001$, $P<0.005$ and $P<0.005$) but high and low MW PCL was poor to impact, and had significantly lower shear strengths ($P<0.005$, $P<0.001$). Fluorostaining showed good cell survival on high MW PLA, high MW PCL and high MW PLGA. These findings were confirmed with WST-1 assays.

High MW PLA as well as high MW PLGA performed well both in mechanical testing and cell compatibility studies. These two polymers are good contenders to produce a living composite for use as substitute human allograft in impaction bone grafting, and are currently being optimised for this use via the investigation of different production techniques and in-vivo studies.

OC41

COMBINED INFLUENCE OF A CONTINUOUS FLOW BIOREACTOR AND BUFFER SYSTEMS ON CHONDROCYTE PROLIFERATION AND

ARTICULAR CARTILAGE TISSUE ACCUMULATION IN LONG TERM CULTURE

AA Khan*[1][2], DC Surrao[2], SD Waldman[2]
[1]Department of Physiology, Anatomy and Genetics, University of Oxford, UK;[2]Department of Chemical Engineering, Queen's University, Canada

Bioreactors used in tissue engineering are mostly batch-fed with media added and removed periodically. Continuous flow bioreactors help increase ECM accumulation and cell proliferation, due to continuous flow of fresh media, thus, maintaining a steady extracellular nutrient environment. In previous work, we found chondrocytes cultured in continuous flow bioreactors with 20mM HEPES, accumulated considerably more matrix than static cultures. Hence, the objective of this study is to determine if NaHCO3 helps maintain a more physiological extracellular pH in the bioreactor, thus, enhancing ECM accumulation.

Cartilaginous tissue constructs were generated from isolated chondrocytes harvested from the metacarpal joints of 12-18 month old calves. Cells were seeded in high-density 3D cultures (2 million cells/construct). Constructs were cultivated in a continuous flow bioreactor, with and without 14 mM NaHCO3 supplemented media, for 5 weeks, at 37 degrees Celsius, 95% relative humidity and 5% CO2. After 5 weeks of culture the tissue weight, thickness, pH and ECM deposition were determined.

From the results obtained (Table 1), it is evident that chondrocytes cultured in the continuous flow bioreactor with 14mM NaHCO3 and 20mM HEPES, proliferated more extensively and produced more ECM than chondrocytes cultured in only 20mM HEPES. Additionally, the NaHCO3 constructs accumulated ECM in both the vertical (thickness) and horizontal (outgrowth) planes. The question then arises, are the effects mediated by improved buffering, or by addition of NaHCO3 itself. There was a significant difference between the pH of media with (pH 7.41) and without NaHCO3 (pH 6.95) supplementation, with no exposure to cells or tissue; when allowed to equilibrate with 5% CO2 at 37 degrees Celsius. However, there was little difference between the media after exposure to cells; after five weeks of culture in the bioreactor (Table 1). Thus, in the bioreactor with bicarbonate present, because of increased cell number and activity, the pH fell 0.54 pH units during the 7 hour residence time in comparison to the bioreactor with no bicarbonate supplementation. With no NaHCO3 supplementation, the extracellular pH of the medium fed to the cells was never above pH 7.0 (Table 1); low pH could account, at least in part, for lower ECM and cell numbers.

OC42

DOES BONE MARROW ASPIRATE AUGMENT BONE FORMATION WITHIN A HYDROXYAPATITE SCAFFOLD?

MJ Coathup*[1], W-J Lo[2], T Edwards[1], GW Blunn[1].
John Scales Centre for Biomedical Engineering, Institute of Orthopaedics and Musculoskeletal Science, Division of Surgery and Interventional Science, University College London, UK [1]. Orthogem Ltd, Biocity, Nottingham, UK [2].

Introduction: This study investigated the binding agent Calcium/Sodium Alginate fibre gel and the addition of autogenic bone marrow aspirate (BMA) on bone growth into a porous HA scaffold implanted in an ovine femoral condyle critical-sized defect. Our hypothesis was that Alginate fibre gel would have no negative effect on bone formation and osteoconduction within the scaffold and that BMA would augment the incorporation of the graft with the surrounding bone at 6 and 12 weeks post implantation.

Methods: 24, 8mm x 15mm defects were filled with either porous HA granules, porous HA granules + Alginate fibre gel (HA putty) or porous HA granules + Alginate fibre gel + BMA (HA putty +BMA) and remained in vivo for 6 and 12 weeks (n=4). 1ml of bone marrow aspirate per cm3 of graft was used. Image analysis quantified bone apposition rates, bone ingrowth, bone-implant contact and quantity of graft. Mann Whitney U tests were used for statistical analysis where $p<0.05$ was considered significant.

Results: Highest bone formation were measured in the 12 week HA putty+BMA group (1.57±0.24(micromillimetres/day). HA granules at 12 weeks encouraged the greatest increase in bone formation (33.56±3.53%). Smaller amounts of bone was measured in the 6 week HA putty+BMA group (8.57±2.86%). Bone formation in the HA group at 12 weeks was significantly higher when compared with the HA putty ($p= 0.043$) and the HA putty+BMA group ($p= 0.043$). At both the 6 and 12 week time point, highest bone-implant contact was seen in the HA granules group (59.34±10.89% and 72.65±3.38% respectively) when compared with both the HA putty ($p=0.018$) and HA putty+BMA ($p=0.047$). Results showed no significant difference in the amount of implant remaining when each group was compared.

Conclusions: Results from this study showed that the inclusion of BMA did not augment bone growth to the scaffold or increase its osteoconductive capacity when combined with Calcium/Sodium Alginate fibre gel. Further research is necessary to optimise Calcium/Sodium Alginate fibre gel when used to bind HA granules and to investigate the effect of BMA with this type of HA alone.

OC43

ENHANCEMENT OF PLA FOR USE IN IMPACTION BONE GRAFTING: THE EFFECT OF PRODUCTION VIA SUPERCRITICAL CO2 DISSOLUTION TO INCREASE POROSITY

ER Tayton*[1], M Purcell[2], A Aarvold[1], JO Smith[1], S Kalra[1], A Briscoe[1],S Fahmy[2], KM Shakesheff[2], S Howdle[2], DG Dunlop[1], ROC Oreffo[1].
[1]Bone and Joint Research Group, University of Southampton, Southampton, UK;[2]School of Chemistry, University of Nottingham, Nottingham,UK

Disease transmission, availability and economic costs of allograft have resulted in significant efforts into finding an allograft alternative for use in impaction bone grafting (IBG). Biotechnology offers the combination of skeletal stem cells (SSC) with biodegradable polymers as a potential solution. Recently polymers have been identified with both structural strength and SSC compatibility that offer the potential for clinical translation.

The aim of this study was to assess whether increasing the porosity of one such polymer via super critical CO2 dissolution (SCD) enhanced the mechanical and cellular compatibility characteristics for use as an osteogenic alternative to allograft in IBG.

High molecular weight PLA scaffolds were produced via traditional (solid block) and SCD (porous) techniques, and the differences characterised using scanning electron microscopy (SEM). The polymers were milled, impacted, and mechanical comparison between traditional vs SCD created scaffolds and allograft controls was made using a custom shear testing rig, as well as a novel agitation test to assess cohesion. Cellular compatibility tests for cell number, viability and osteogenic differentiation using WST-1 assays, fluorostaining and ALP assays were determined following 14 day culture with SSCs.

SEM showed increased porosity of the SCD produced PLA scaffolds, with pores between 50-100 micrometres. Shear testing showed the SCD polymer exceeded the shear strength of allograft controls ($P<0.001$). Agitation testing showed greater cohesion between the particles of the SCD polymer ($P<0.05$). Cellular studies showed increased cell number, viability and osteogenic differentiation on the SCD polymer compared to traditional polymer ($P<0.05$) and allograft ($P<0.001$).

The use of supercritical C02 to generate PLA scaffolds significantly improves the cellular compatibility and cohesion compared to traditional non-porous PLA, without substantial loss of mechanical shear strength. The improved characteristics are critical for clinical translation as a potential osteogenic composite for use in impaction bone grafting.

OC44

PROTECTION OF ARTICULAR CARTILAGE AGAINST DRYING BY GLUCOSE-SALINE OR SYNOVIAL FLUID

BT McLintock[1], CE Banfield[1], AK Amin[2], AC Hall*[1]
[1]Centre for Integrative Physiology, University of Edinburgh, UK;[2]Department of Orthopaedic and Trauma Surgery, Royal Infirmary of Edinburgh, UK

Saline (0.9%) is typically used to rinse joints during osteo-articular surgery. It is not unusual for cartilage to then be exposed to the air of the operating theatre for 1-2hrs, which can lead to chondrocyte death. We have compared the survival of in situ chondrocytes within bovine cartilage which has been rinsed in various solutions or simply drained of synovial fluid (SF) and then allowed to dry, to identify approaches that could reduce chondrocyte death arising from cartilage drying.

Metacarpophalangeal joints from 3yr-old cows were opened under aseptic conditions. The joints were then (a) rinsed with saline (Baxter's Healthcare, Newbury), (b) rinsed with saline+glucose (20mM; both 300mOsm) or (c) drained of SF, and allowed to dry at room temperature. Full depth cartilage explants were taken after 2hrs, placed into Dulbecco's modified Eagle's medium and incubated with CMFDA (5-chloromethyl-fluorescein diacetate; 10microM) and propidium iodide (10microM) for the identification/quantification of living and dead cells respectively by confocal scanning laser microscopy and image analysis.

After 2hrs, the appearance and properties of the cartilage of the drying joints were clearly different. Saline-rinsed cartilage was dark purple and appeared dull with the cartilage difficult to sample. However when the rinsing solution was saline+glucose, or when joints were drained of SF, the cartilage was almost identical to the freshly-opened joint with a pearly-blue, shiny appearance, and cartilage sampling was easy.

Chondrocyte death was markedly increased in saline rinsed/dried joints after 2hrs (21±9% cell death). In contrast, there was no significant ($P>0.05$) death in saline+glucose rinsed/dried (2±1%) or SF-drained joints (3±2%;means±s.e.m.;n=5). The loss of cartilage wet weight over 2hrs (time=0 taken as 100%) was almost identical between cartilage rinsed in saline (73.6±1.6%), saline + glucose (78.6±1.1%) or SF (75.0±0.2%; data means±s.d.;n=2).

These results suggest that it was not the loss of water per se during cartilage drying that was the key determinant of chondrocyte viability. As chondrocytes are normally anaerobic, the rise in cartilage pO2 which occurs during exposure to air could have a deleterious effect on cell viability however the presence of glucose or SF protects through an anti-oxidant effect.

OC45

METAL ON METAL TOTAL HIP REPLACEMENT: INFLUENCE OF HEAD SIZE UNDER ADVERSE HIP SIMULATOR CONDITIONS

M AL-Hajjar*, J Fisher, S Williams, JL Tipper, LM Jennings
Institute of Medical and Biological Engineering, School of Mechanical Engineering, University of Leeds, Leeds, UK

In vitro the introduction of microseparation and edge loading to hip simulator gait cycle has replicated clinically relevant wear rates and wear mechanisms in ceramic-on-ceramic bearings[1], and elevated the wear rates of MoM surface replacements (SR) to levels similar to those observed in retrievals[2]. The aim was to assess the wear of two different sized MoM total hip replacement bearings under steep cup inclination angles and adverse microseparation and edge loading conditions.

Two tests were performed on the Leeds II hip joint simulator using two different size bearings (28mm and 36mm). Cups were mounted to provide inclination angles of 45 degrees (n=3) and 65 degrees (n=3). The first three million cycles were under standard gait conditions. Microseparation and edge loading conditions as described by Nevelos et al [1] were introduced to the gait cycle for the subsequent three million cycles. The lubricant was 25% new born calf serum. The mean wear rates and 95% confidence limits were determined and statistical analysis was performed using One Way ANOVA.

Under standard gait conditions, when the cup inclination angle increased from 45 degrees to 65 degrees, the wear of size 28mm bearing significantly ($p=0.004$) increased by 2.7-fold, however, the larger bearings did not show any increase in wear ($p=0.9$). The introduction of microseparation conditions resulted in a significant ($p=0.0001$) increase in wear rates for both bearing sizes under both cup inclination angle conditions. Under microseparation conditions, the increase in cup inclination angle had no influence on the wear rate for both bearing sizes (Figure 1).

With larger bearings, head-rim contact occurs at a steeper cup inclination angle providing an advantage over smaller bearings. The introduction of edge loading and microseparation conditions resulted in a significant increase in wear rates for both bearing sizes. The wear rates obtained in this study under combined increased cup inclination angle and microseparation were half of those obtained when SR MoM bearings were tested under similar adverse conditions[2]. This study shows the importance of prosthesis design and accurate surgical positioning of the head and acetabular cup in MoM THRs.

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OC46

INTERVERTEBRAL DISC FISSURES PROVIDE A LOW-PRESSURE, CHEMICALLY CONDUCIVE MICRO-ENVIRONMENT FOR NERVE IN-GROWTH

M Stefanakis*[1], J Luo1 [2], P Pollintine [1], T Ranken [1], J Harris [1], P Dolan[1], M A Adams[1]
[1]Centre for Comparative and Clinical Anatomy, University of Bristol, Bristol U.K; [2]Roehampton University

Introduction: The feature of disc degeneration most closely associated with pain is a large fissure in the annulus fibrosus. Nerves and blood vessels are excluded from normal discs by high matrix stresses and by high proteoglycan (PG) content. However, they appear to grow into annulus fissures in surgically-removed degenerated discs. We hypothesize that anulus fissures provide a micro-environment that is mechanically and chemically conducive to the in-growth of nerves and blood vessels.

Methods: 18 three-vertebra thoraco-lumbar spine specimens (T10/12 to L2/4) were obtained from 9 cadavers aged 68-92 yrs. All 36 discs were injected with Toluidine Blue so that leaking dye would indicate major fissures in the annulus. Specimens were then compressed at 1000 N while positioned in simulated flexed and extended postures, and the distribution of compressive stress within each disc was characterised by pulling a pressure transducer through it in various planes. After testing, discs were dissected and the morphology of fissures noted. Reductions in stress in the vicinity of fissures were compared with average pressure in the disc nucleus. Distributions of PGs and collagen were investigated in 16 surgically-removed discs by staining with Safranin O. Digital images were analysed in Matlab to obtain profiles of stain density in the vicinity of fissures.

Results: Fifteen circumferential or radial annulus fissures were identified. Focal compressive stress within the fissures was lower than nucleus pressure, by 11-26% in normal discs, and by 47-63% in degenerated discs, depending on posture. Stress reductions within fissures were inversely related to average nucleus pressure ($p<0.05$). The edges of fissures were usually depleted of PGs, leaving a collagenous scaffold, and PG stain density remained depleted (less than 65% of the disc average) for an average 450 micrometers from the fissure edge.

Discussion: Matrix compressive stresses are particularly low within annulus fissures if the disc nucleus is degenerated and decompressed. Nucleus pressure normally tensions the anulus, and loss of this support mechanism allows focal decompression within fissures. This in turn may allow focal swelling and PG loss. Results support the hypothesis: annulus fissures are conducive to nerve and blood vessel in-growth.

OC47

THE EFFECT OF HEAT GENERATED BY BONE CEMENT ON THE VIABILITY OF HUMAN MESENCHYMAL STEM CELLS

Y Reissis*, E Garcia, J Hua, G Blunn
University College London, London, UK

Impaction allograft using cement is commonly used in revision surgery for filling bone defects and provides a load bearing interface. However, the variable regeneration of new bone within the defect makes clinical results inconsistent. Previous studies showed that addition of mesenchymal stem cells (MSCs) seeded on allograft can enhance bone formation in the defect site. The purpose of this study is to test the hypothesis that heat generated during cement polymerization will not affect viability of the human MSCs.

The temperatures and durations were taken from previous studies that recorded the maximum temperature generated at the bone-cement interface. Temperatures of below 30 degrees Celsius to over 70 degrees Celsius have been detected and the duration of elevated temperature varies from 30 seconds to 5 minutes. In this study the viability of MSCs cultured at different temperatures was assessed. Ten groups were studied with three repeats (Table 1). A control group in which cells were cultures normally was used. Culture medium was heated to the required temperature and added to the cells for the required duration. The metabolism of MSCs was measured using the alamar Blue assay, cell viability was analysed using Trypan Blue and cell apoptosis and necrosis were tested using Annexin V and Propidium Iodide staining.

Results showed that cell metabolism was not affected with temperatures up to 48 degrees Celsius for periods of 150s, while cells in the 58 degrees Celsius group eventually died (Fig.1). Similar results were shown in Trypan Blue analysis (Fig.2). When comparing the group of cells heated to 48 degrees Celsius for 150s with the control group for apoptosis and necrosis, no significant difference was observed.

The study suggests that human MSCs seeded to allograft can be exposed to temperatures up to 48 degrees Celsius for 150s, which covers many of the situations when cement is used. This indicates that the addition of mesenchymal stem cells to cemented impaction grafting can be carried out without detrimental effects on the cells and that this may increase osteointegration.

OC48

ULTRASTRUCTURAL CHARACTERISATION OF COBALT/CHROMIUM METAL NANOPARTICLES IN MACROPHAGES AND CONSEQUENT CYTOTOXICITY

Zhidao Xia*[1], David Murray[2]
[1]Centre for Nanohealth, Institute of Life Science, College of Medicine, Swansea University, Singleton Park, Swansea [2]Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, NOC, Headington, Oxford

Metal and their alloys have been widely used as implantable materials and prostheses in orthopaedic surgery. However, concerns exist as the metal nanoparticles released from wear of the prostheses cause clinical complications and in some cases result in catastrophic host tissue responses. The mechanism of nanotoxicity and cellular responses to wear metal nanoparticles are largely unknown. The aim of this study was to characterise macrophage phagocytosed cobalt/chromium metal nanoparticles both in vitro and in vivo, and investigate the consequent cytotoxicity. Two types of macrophage cell lines, murine RAW246.7 and human THP-1s were used for in vitro study, and tissues retrieved from pseudotumour patients caused by metal-on-metal hip resurfacing (MoMHR) were used for ex vivo observation. Transmission electron microscopy (TEM), scanning electron microscopy (SEM) in combination with backscatter, energy-disperse X-ray spectrometer (EDS), focused ion beam (FIB) were employed to characterise phagocytosed metal nanoparticles. Alamar blue assay, cell viability assays in addition to confocal microscopy in combination with imaging analysis were employed to study the cytotoxiticy in vitro. The results showed that macrophages phagocytosed cobalt and chromium nanoparticles in vitro and the phagocytosed metal particles were confirmed by backscatter SEM+EDS and FIB+EDS. these particles were toxic to macrophages at a dose dependent manner. The analysis of retrieved tissue from revision of MoMHR showed that cobalt/chromium metal nanoparticles were observed exclusively in living macrophages and fragments of dead macrophages, but they were not seen within either live or dead fibroblasts. Dead fibroblasts were associated with dead and disintegrated macrophages and were not directly in contact with metal particles; chromium but not cobalt was the predominant component remaining in tissue. We conclude that as an important type of innate immune cells and phagocytes, macrophages play a key role in metal nanoparticles related cytotoxicity. Metal nanoparticles are taken up mainly by macrophages. They corrode in an acidic environment of the phagosomes. Cobalt that is more soluble than chromium may release inside macrophages to cause death of individual nanoparticle-overloaded macrophages. It is then released into the local environment and results in death of fibroblasts and is subsequently leached from the tissue.

OC49

DISCORDANCE BETWEEN CORTICAL AND TRABECULAR BONE PHENOTYPE HIGHLIGHTS THE ROLE OF LOCAL VERSUS CIRCULATING IGF-1 IN THE SOCS2 NULL MOUSE

R Dobie*[1], C Huesa[1], RJ van't Hof[2], VE MacRae[1], SF Ahmed[3], C Farquharson[1]
[1]Roslin Institute & R(D)SVS, University of Edinburgh, UK;[2]Centre for Molecular Medicine, University of Edinburgh, UK; [3]Bone and Endocrine Research Group, University of Glasgow, UK

Suppressor of Cytokine Signalling 2 (SOCS2) tightly regulates postnatal growth through its actions on the GH/IGF-1 pathway. SOCS2 knockout mice do not have raised circulating IGF-1 and are characterised by enhanced body size and bone length. However the trabecular architecture and cortical geometry of the bones have yet to be fully investigated. Further to this, the bone mechanical properties remain undefined. In order to better appreciate the role of SOCS2 in juvenile and adult male and female mice, we have conducted an in depth analysis of the bone phenotype.

Micro-CT was carried out on the tibia of wild-type (WT) and SOCS2 knockout (KO) mice, at 6-weeks (juvenile) and 4-months (adult) of age. Analysis enabled detailed comparisons to be made between the trabecular and cortical macroarchitecture, and allowed for the calculation of the mineral density of the bone tissue. 3-point-bending was also completed to investigate the mechanical properties.

A significant increase in body weight was observed in all KO mice ($P<0.05$). All KO mice had increased trabecular BV/TV compared to their age and sex matched WT controls which is a likely consequence of increased trabecular thickness ($P<0.05$). All KO mice had increased tibial periosteal surface area ($P<0.05$), however the cortex was thicker only in juvenile and adult KO male mice. The thicker cortex of the male KO mice tibia was associated with increased work to failure and maximum load. At 6 weeks of age there was no difference in the mineral density of the bone tissue in either gender, however at 4 months both male and female KO mice had a significant decrease of mineral density in the cortical bone ($P<0.05$).

The increased bone size and mass are consistent with the known anabolic effects of local, skeletal, IGF-1 and these effects are age and sex specific. The fall in mineral density of cortical bone reflects the importance of circulating IGF-1 on cortical bone development. The SOCS2 KO mouse represents a valid model for studying the effects of GH and IGF-1 on bone.

OC50

SPIROSTOMUM AMBIGUUM: A PROTOZOAN MODEL FOR PRIMORDIAL MUSCULOSKELETAL EXCHANGE?

PE Garner*[1,2], V Fallon[1], JE Aaron[1]

[1]IMSB, FBS, University of Leeds, UK; [2]IMBE, University of Leeds, UK

The skeleton is responsive to mechanical usage, yet the basis for its remarkable sensitivity remains uncertain. Abundant osteocytes seem central. Their cytoplasmic syncitium is pervasive and well placed to bridge the gap between mechanical signal transduction and cellular. However, its calcified entombment limits accessibility, while isolation or manipulation may alter its specific threshold characteristics. Insight into the rudiments of musculoskeletal exchange may be found in certain protozoa from which the metazoan pathway apparently evolved (Pautard 1960, 1970; Ruffalo 1978). In particular is the organism Spirostomum ambiguum (a cigar-shaped creature visible to the naked eye) which fabricates and accumulates calcium phosphate particles resembling those found in bone. Moreover, their intracellular, golgi-directed synthesis (Fallon, Garner and Aaron, in press) is determined by their active life-cycle. This modulates between a free-swimming state when calcified particles are few and a burrowing stage when calcified particles are many. Thus when the mineral of cultured S. ambiguum was labelled with the fluorochrome tetracycline the green fluorescence intensity (AU), mapped using laser confocal microscopy, recorded a high mineral level in the burrowing animals (138.0 + SD4.0) compared with the free-swimmers (89.7 SD 3.3). Similarly when the live organisms were transfected with a GFP construct (Fallon, 2006) the resulting mannosidase II enzyme, as an expression of Golgi activity, differed significantly ($p<0.0001$) between tunnelling (104.6 SD 2.7) and free-swimming (74.5 6.7) by the two-sample t-test. Also it was observed that the distribution of the calcified particles was not random. A proportion related in disposition to a regular and well-defined pattern of contractile muscle myonemes, the fibres of which were arranged longitudinally within the high stress burrowers in contrast to their transverse alignment in the low stress swimmers. The capacity exhibited by this animal model not only to package bone-like mineral in response to changing environmental pressures, but also to relate them to their intracellular contractile elements may suggest an early integrated musculoskeletal system that substantially predated the vertebrates which eventually exploited this major advantage. The protozoan model described may therefore serve as a valuable tool for future fundamental investigation of osteocyte ancestry, mechanotransduction, perception and response.

OC51

NOVEL REGULATION STEPS IN PARATHYROID RECEPTOR ACTIVATION

GO Richards*[1], DJ Roberts[1], TM Skerry[1]

The Mellanby Centre for Bone Research, Department of Human Metabolism, University of Sheffield, UK

The mechanisms by which cells perceive and respond to their environment is fundamental to tissue, organ and organism function. The majority of cell perception of local and systemic stimuli is performed by GPCRs and so it is unsurprising that GPCR modulating agents form the majority of pharmaceutical therapies. GPCRs elicit their intracellular actions by activation of a hetrotrimeric G-protein The subtype of G-protein activated dictates second messenger activation so that Gs activates cAMP, Gi inhibits cAMP and Gq activates Ca2+. However, traditional views of a single receptor responding predominantly to a single ligand by regulating intracellular calcium and cAMP activation fail to explain many of the subtleties of cell responses to stimuli. Parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP) both activate the same PTH1 receptor (PTH1R), yet it is unknown how the two different ligands can exert different effects.

Here we demonstrate a potential mechanism by which the two different ligands can bind to the same receptor to induce different patterns of G-protein activation, and that the same ligand can cause a different pattern of G-protein activation in the presence of a Receptor Activity Modifying Protein (RAMP).

Specifically we show in COS-7 cells transfected with a range of receptor and RAMP constructs and using a scintillation proximity assay for G-protein activation that PTH induced a 39% greater maximal activation of the G alpha s and a 67% increase G alpha i activation compared with PTHrP ($p<0.05$ in 3 independently replicated studies for each combination each n=3). Furthermore the association of the PTH1 receptor with RAMPs 2 alters G-protein induction by PTH. RAMP2 increased G alpha s activation of the PTH1R by PTH by 58% and G alpha i 37% compared with receptor alone. However the association of the RAMP with the receptor did not alter the affinity of the ligand for the receptor.

These findings illustrate a level of complexity of ligand/receptor/cell activation which provides potential therapeutic benefits by allowing selection of modulatory actions beyond current simple agonists and reverse agonists.

OC52

ACTIVE SHAPE MODELLING FOR HIP FRACTURE PREDICTION

SR Goodyear*[1],RJ Barr[1],K Yoshida[1],RM Aspden[1],IR Reid[2],DM Reid[1],JS Gregory[1]

[1]Musculoskeletal Programme, Division of Applied Medicine, University of Aberdeen, UK;[2]Department of Medicine, University of Auckland, NZ

Active shape modelling (ASM) has been shown to be of similar value to BMD in predicting hip fractures and, together with BMD, to improve prediction accuracy. Use of ASM to identify individuals at the highest risk of hip fracture may allow better targeting of expensive treatments to the neediest individuals.

The outline of the hip joint was described using ASM (proximal femur, acetabulum and parts of the pelvis) in DXA (Lunar Expert) images. The coordinates of 83 landmark points were found semi-automatically in each image and PCA used to reduce these variables to a few modes of variation. For each image, a score for each mode was calculated.

The model was built from baseline DXA images of women entering a calcium supplementation trial performed in Auckland, NZ. 21 hip fracture subjects were matched by age, height, weight and BMD with 63 subjects who did not fracture. A second control group (n=63) was selected at random.

In the matched comparison modes 6 and 10 were significant predictors of hip fracture (mode 6 OR = 1.68, 95% CI = 1.00-2.80, P = 0.048, mode 10 OR = 2.24, 95% CI = 1.22-4.10, P = 0.009), and together gave a better prediction, than either mode alone (AUC mode 6 = 0.64, mode 10 = 0.70 and together = 0.73).

In the random comparison age, BMD (T-score) and mode 1 were significant predictors of fracture (Table 1) with age giving the best prediction (AUC = 0.72). After adjustment for age only mode 10 remained a significant predictor. In models including BMD, T-score was a less significant predictor than mode 1 and gave no predictive value when combined with mode 10.

These results show ASM can generate modes that are predictive of hip fracture in comparisons with matched controls and controls selected randomly from the whole study population. In this cohort, shape gave as good a prediction of fracture as BMD (T-score), by AUC, and in a combined model was a more significant prediction variable than BMD. Shape, therefore, provides a valuable additional contribution to predicting individuals at high risk of hip fracture.

OC53

BONE IN VITRO 3D OSTEOBLAST-OSTEOCYTE CO-CULTURE MODEL

M Vazquez*[1], BAJ Evans[3], J Ralphs[2], D Riccardi[2], DJ Mason[2]

[1]Arthritis Research UK Biomechanics and Bioengineering Centre, School of Biosciences, Cardiff University, Museum Avenue, Cardiff, CF10 3AX; [2]Division of Pathophysiology and Repair, School of Biosciences, Cardiff University, Museum Avenue, Cardiff, CF10 3AX, UK; [3]Department of Child Health, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, UK.

Background: Osteoporosis occurs when the activities of bone cells become unbalanced leading to increased bone remodelling and weakening of bones. Anabolic therapies are limited, necessitating further investigation of the mechanisms that regulate bone formation. Normal mechanical loading potently induces bone formation via effects on osteocytes. Current investigations of mechanical loading of bone do not reflect the interactions of the cells within it, with most focusing on mechanical loading of osteoblasts in monolayers. Of the 3D models that do exist, none elucidate the osteoblast-osteocyte interactions that regulate mechanically-induced bone formation. We are developing a novel in vitro 3D co-culture model of bone to investigate osteoblast-osteocyte interactions (1) and the role of glutamate, calcium and adenosine signalling in bone mechanotransduction.

Methods: MLO-Y4 cells (1.5x10^6 cells/ml) were incorporated into acid-soluble rat tail tendon type I collagen (2 mg/ml in alpha MEM, pH7.4) gels and MC3T3-E1(1.0x10^5 cells/well) layered on top and cultured at 37C in SMEM (1 mM CaCl2, 2 mM Glutamax, 50 microgram/ml ascorbic acid, 5 % dialysed FBS) for 1 week. Co-cultures were fixed with 1 % paraformaldehyde, infiltrated with OCT, cryosectioned and labelled with phalloidin and DAPI to assess cell morphology, ethidium homodimer and DAPI to assess cell viability or immunostained using anti-connexin 43 antibody to assess cell connectivity. Osteoblast and osteocyte phenotype were determined by RT-PCR of RNA extracted (Trizol) separately from surface osteoblasts and encased osteocytes.
Results: Preliminary data show co-cultures survive, for at least one week, with osteocyte cell death within gels (18 +/- 2.16 %, n=3) comparable to monolayer cultures (2). MLO-Y4 and MC3T3-E1 cells maintain their morphology, form a network through connexin 43, and express osteocyte and osteoblast phenotypic markers respectively, and components of the glutamate, calcium and adenosine signalling pathways.
Conclusion: We have established a mouse osteoblast-osteocyte 3D co-culture system which can be used to investigate co-ordination of mechanical signalling through glutamate, calcium and adenosine delineating their roles in mechanically-induced bone formation.
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OC54

NANOTOPOGRAPHY-INDUCED OSTEOGENIC DIFFERENTIATION OF HUMAN EMBRYONIC AND ADULT SKELETAL STEM CELLS

E Kingham*[1], MP Tsimbouri[2], N Gadegaard[3], MJ Dalby[2], ROC Oreffo[1]
[1]Human Development and Health, University of Southampton, Southampton United Kingdom; [2]Centre for Cell engineering, Faculty of Biological & Life Sciences, Joseph Black Building, University of Glasgow, Glasgow, United Kingdom; [3]Centre for Cell engineering, Department of Electronics and Electrical Engineering, Rankine Building, University of Glasgow, Glasgow, United Kingdom

The efficient production of stem cell-derived cell types will be of significant medical and research benefit. Moreover, the use of nanopatterned substrates as biomimetic surfaces for stem cells provides a unique, physical approach to overcome the risks associated with chemical induction and genetic manipulation.
Using polycarbonate substrates with nanoscale topography (structural features of 35-120nm), we have investigated the influence of nanopits with ordered geometries on human embryonic stem cell and human adult skeletal stem cell fate.
Adult skeletal stem cells responded differentially to varying geometries and dimensions of nanopit. Notably, a disordered square pattern, displaced by 50nm, induced the expression of osteogenic markers, osteopontin and bone sialoprotein in the absence of chemical osteogenic factors. PCR arrays and immunofluorescence were used to further characterise the cell types resulting from culture on defined nanotopographies and indicated osteogenic-directed differentiation highlighting therapeutic opportunities for in vitro directed differentiation and for the surface of orthopaedic implants to improve osseointegration.
Embryonic stem cells responded to disordered square nanotopography by a loss of expression of self-renewal markers Nanog, Oct4, Sox2, TRA-1-60, SSEA-3 and SSEA-4 and by enhancement in expression of skeletal stem cell-associated genes and markers indicative of early osteogenic progenitor cells CD63, ALCAM, Runx2, osteonectin and BMP4.
To investigate potential epigenetic mechanisms for nanotopography induced stem cell transcriptional changes, the methylation status of the osteocalcin gene promoter region was examined. The level of osteocalcin promoter methylation could be linked to the developmental stage of cell-types used but nanotopography did not induce hypomethylation.
A nanomaterials approach offers new strategies to overcome current issues of stem cell attachment, expansion, lineage specification and directed differentiation in regenerative medicine. The current studies demonstrate defined nanoscale patterns can directly modulate differentiation of human embryonic and adult stem cells and offer an innovative approach to guide osseointegration, improve healing and implant longevity in the orthopaedic environment with broad application in regenerative medicine.

CLINICAL CASES

CC1

FIBROBLAST GROWTH FACTOR-23: STRONG ASSOCIATION WITH THE RENAL FANCONI SYNDROME IN A PATIENT WITH ONCOGENIC OSTEOMALACIA

AGW Norden* [1], RJC Laing [2], RJ Unwin [3], OM Wrong [3], AJ Crisp [4]
[1]Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK;[2] Department of Neurosurgery, Addenbrooke's Hospital, Cambridge, UK;[3] Department of Renal Medicine, University College London, London, UK;[4] Department of Rheumatology and Metabolic Bone Disease, Addenbrooke's Hospital, Cambridge, UK

Oncogenic Osteomalacia is associated with secretion of Fibroblast Growth Factor-23, (FGF-23) and other phosphaturic peptides by a tumour usually of mesenchymal origin. Hypophosphataemia, a consequence of the reduced renal phosphate threshold caused by these peptides, and clinical osteomalacia, is relatively common. Rarely, glycosuria and aminoaciduria as features of the renal Fanconi syndrome, (Lancet 1972;1 No.7746:353-4 and Amer. J. Medicine 1985;78:708-10), have also been found. Tubular proteinuria, a predominantly low molecular weight proteinuria, and usually a cardinal feature of Fanconi syndrome, has not specifically been reported before.
We investigated a 78 year old female patient with hypophosphataemia and oncogenic osteomalacia, known to have high levels of plasma FGF-23 pre-operatively, and associated with marked renal Fanconi syndrome including tubular proteinuria, glycosuria and aminoaciduria. Tubular proteinuria was shown by grossly elevated urinary excretion of retinol-binding protein (RBP) and Beta-2-microglobulin relative to albumin. Previous management included parathyroidectomy and oral phosphate and 1-alpha-hydroxy VitaminD. 18F-Deoxyglucose PET scanning and MRI demonstrated a paraspinal tumour at the level of T9 vertebral body and complete macroscopic resection of the tumour was performed. We measured plasma FGF-23, the renal Transport Maximum of Phosphate/Glomerular Filtration rate, TmP/GFR, (to estimate renal phosphate threshold), urine RBP and albuminuria (to measure tubular proteinuria), glycosuria and aminoaciduria. All 5 measurements except aminoaciduria, were made on some 15 occasions, pre-, peri- and post-operatively.
The resected tumour comprised primitive mesenchymal cells without evidence of malignancy. Plasma FGF-23 measured by a C-terminal assay (Immunotopics Inc.) fell from a pre-operative level of 4500 to 314 Relative Units/L within 24 hours of tumour resection and was undetectable by 22 days. The estimated renal threshold for plasma phosphate reabsorption, (Ref. Range 1.03+/-0.2), rose progressively from 0.24 mmol/L preoperatively to 0.75 (4 days after resection) and 1.44 (22 days) with normalisation of plasma phosphate concentrations. Full resolution of the Fanconi syndrome was shown by abolition of tubular proteinuria, glycosuria and aminoaciduria which closely followed plasma FGF-23 levels.
In this patient with Oncogenic Osteomalacia, either FGF-23, or other humoural factors highly correlated with FGF-23, caused renal Fanconi syndrome.

CC2

CORD COMPRESSION RESULTING FROM FIBROUS DYSPLASIA (FD) OF THE SPINE : A CASE REPORT

I Scott*[1],J Lucas[2], G Gnanasegaran[3], C Silve[4], I Fogelman[3,1],G Hampson[1]
[1]Osteoporosis clinic, Guy's Hospital, London UK, [2]Orthopaedics, Guy's Hospital, London UK, [3]Nuclear Medicine, Guy's Hospital, London UK, [4]Molecular Genetics Laboratory, Hospital Bichat, Paris, France

The patient was a 59 year old man who was diagnosed with fibrous dysplasia (FD) 20 years previously. CT scan at the time showed involvement of the 3rd, 5th and 8th rib only. He remained asymptomatic until recently when he started getting pain in his chest and upper back. Neurological examination was normal. Repeat CT and MRI scan confirmed progression of the bone lesions with grossly expanded sclerotic bone and lytic lesions involving particularly T2, T4 and T5. There was complete destruction of the vertebral body of T3 with bulging of the FD lesions into his spinal canal at T3. There was also involvement of his ribs consistent with polyostotic FD. 99TC-MDP bone scan showed intense uptake involving the r-sided ribs and upper thoracic spine. An 18 F FDG PET scan showed increased FDG uptake in the expansile and lytic lesions of the spine (cervical and thoracic spine) and right ribs. The appearances were in keeping with metabolically active disease. He was referred to the metabolic bone clinic for treatment with i.v bisphosphonate. Laboratory findings confirmed increased bone turnover (alkaline phosphatase : 209 (35-129 IU/L), P1NP : 172 (28-80 ug/L) and hypophosphataemia (0.6 mmol/L). However, bisphosphonate treatment was delayed due to a planned knee operation. He developed progressive myelopathy with difficulty in walking and sensory loss at T5 sensory level and required emergency admission. MRI confirmed encroachment upon the spinal canal causing cord compression. He underwent urgent T4/T5 thoracic laminectomy and cord decompression. Histology showed bone fragments containing irregular trabeculae of woven bone and an aneurysmal bone cyst containing osteoclast giant cells. There was no evidence of malignancy. Post-operatively he made an uneventful recovery and was treated medically with i.v Zoledronate and Calcium/vitamin D supplements with improvement in his bone pain. Mutation analysis of his peripheral blood cells did not show any mutation in the GNAS gene at position R201H.
FD is a skeletal disorder characterised by abnormal fibro-osseous tissue. Skeletal involvement leads to pain, deformity and fractures. Treatment with bisphosphonate reduces pain and bone turnover. The molecular cause is due to post-zygotic activating mutations in the GNAS gene.

POSTERS

P001

AUTOLOGOUS OSTEOCHONDRAL TRANSPLANTATION OF THE TALUS - REGIONAL AND LOCAL CONTACT MECHANICS AND A GRAFT HEIGHT ANALYSIS - A BIOMECHANICAL STUDY

AM Fansa, CD Murawski*, CW Imhauser, JT Nguyen, S Schafmeister, JG Kennedy

Hospital for Special Surgery, New York, NY, USA

This study was designed using robotic technology to biomechanically evaluate and quantify the changes in contact mechanics on the talar dome after the creation of an osteochondral defect and implantation of an osteochondral graft.

Ten fresh-frozen cadaveric lower limb specimens were used for this study. Specimens were loaded using a six-degrees-of-freedom robotic arm and contact mechanics were simultaneously measured using a standard ankle joint pressure sensor. An 8-millimeter osteochondral defect was created at the centromedial aspect of the talar dome. An autologous osteochondral graft from an ipsilateral knee was then transplanted to the defect site in the most congruent position possible. Regional contact mechanics were analyzed across the talar dome as a function of the defect and repair conditions and compared to the intact ankle. Local contact mechanics at the peripheral rim of the defect and at the graft site were also analyzed and compared to the intact condition. A three-dimensional laser scanning system was used to determine the graft height differences relative to the native talus.

The creation of a centromedial defect significantly decreased mean force, mean pressure and peak pressure on the medial region of the talus ($p<0.05$). Implanting an osteochondral graft in the defect site restored mean force, mean pressure and peak pressure on the medial region of the talus to intact levels ($p>0.05$). Mean force, mean pressure and peak pressure on the peripheral rim of the defect were also restored to intact levels. The posterior region of the graft sustained a significant increase in force, mean pressure and peak pressure relative to the intact condition ($p=0.024$, 0.047, 0.054, respectively). The mean graft height difference of the overall population was -0.2 ± 0.3 millimeters (range -1.00 mm to 0.40 mm).

Implanting an osteochondral graft at the centromedial aspect of the talus resulted in the restoration of contact mechanics on the talar dome. However, certain local regions of the graft were subjected to significant changes in pressures despite a trained orthopaedic surgeon placing the graft in the most congruent position possible. Further investigation is warranted to optimize intraoperative methods of obtaining perfect articular congruency.

P002

HOW MUCH CASUAL SUNSHINE IS ENOUGH FOR ADEQUATE VITAMIN D STATUS

JL Berry*[1], LE Rhodes [2], R Kift [3], AR Webb [3]

[1]Vitamin D Research Laboratory, University of Manchester School of Developmental Biomedicine, Manchester Royal Infirmary, Manchester Academic Health Science Centre, Manchester M13 9WL; [2]Photobiology Unit, Dermatological Sciences, School of Translational Medicine, Salford Royal NHS Foundation Hospital, [3]School of Earth Atmospheric and Environmental Sciences, University of Manchester, Manchester M13 9PL, UK

It is assumed that healthy, ambulatory fair-skinned adults will gain their vitamin D requirements from sun exposure, and thus the reference nutrient intake for healthy white non-elderly adults in the U.K. is currently zero. The Health Protection Agency advises that brief exposures to summer sunlight containing the requisite UVB are sufficient to maintain vitamin D status but this refers only to the requirement of avoiding severe vitamin D deficiency [25-hydroxyvitamin D (25OHD) <5 ng/mL (12.5 nmol/L)]. We have recently reported on the effects of simulated summer's sun exposures on the vitamin D status of white Caucasian adults using a carefully characterised irradiation cabinet. The irradiation regime (1.3 SED given 3 times weekly for 6 weeks) significantly raised 25OHD levels, with 90% of volunteers reaching sufficiency, defined as a circulating 25OHD (> or =)20 ng/mL (50 nmol/L). Although this study provided fundamental information necessary to judge the effectiveness of the irradiation regime, it is standardised and artificial in nature.

Using a radiative transfer model SMARTS 2.95 we now show how our results can be translated into everyday situations. This model assumes subjects wear modest summer clothing (exposing 35% of the body surface) and that exposure is either side of local solar noon. We considered a standing body, randomly orientated with respect to the sun and compared this to the experimental horizontal body in the equivalent radiation field. For our midsummer, noontime, Manchester situation (measured at a solar elevation of 60 degrees) a vertical person, randomly oriented towards the sun, would acquire their vitamin D dose in approximately 33 minutes, while at 13 minutes per side, the horizontal subject required approximately 26 minutes. When noon, solar elevation was at 45 degrees (ie lunchtime in April/August) the times needed increased to 49 minutes (vertical) and 44 minutes (horizontal).

We conclude that UV doses equivalent to those found experimentally can be acquired naturally and in a realistic time period (eg a lunch-time walk in full sunlight and suitably clothed to expose sufficient skin). However, considerable inter-individual variation in 25OHD response occurs following exposure both to a real and simulated summer, requiring further investigation.

P003

CHANGES IN MOUSE GAIT MAY PREDICT OA-LIKE LESION PROGRESSION

RL de Souza*[1,2], B Poulet [1], AA Pitsillides [1]

[1]Department of Basic Siences, Royal Veterinary College, UK; [2]Department of Veterinary Clinical Sciences, Federal University of Mato Grosso, Brazil

INTRODUCTION: Loss of joint function is only exploited in osteoarthritis (OA) once severe impairment is apparent. Animal models allow for lesion induction and serial OA progression measures. We recently described an adjustable non-surgical loading model for generating focal cartilage lesions in only the lateral femur joint compartment, in which regimes can be adjusted so that these either do or do not progress spontaneously. Herein, we use ventral plane videographic treadmill gait analysis to determine whether gait changes can be used to discriminate between stable and spontaneously progressing lesions, induced by these two loading regimes.

METHODS: Animals encountered normal conditions, except during loading (9N, 40 cycles, 0.1 Hz, 10 sec/ cycle) which was applied to right knees in two groups (n=8) of 8-week-old male CBA mice: i) loaded once; ii) loaded 3 times/week for 2 weeks. Gait (including: brake, propel, stance, stride, stride length, stride frequency, steps and paw area) was assessed 3 times/week for 2 weeks in each mouse using a DigigaitTM treadmill. Thereafter, mice received 5mg/kg carprofen for analgesia and gait analysis repeated on 3 further alternate days.

RESULTS: The two loading regimes produced virtually identical gait modifications with delayed onset (apparent on day 3) which remained unchanged for 2 weeks; mice loaded once only showed modified contralateral limb use, but those loaded multiply exhibited additional ipsilateral front limb modifications; no changes in gait were observed in loaded limbs. Intriguingly, the two regimes produced distinct responses to analgesia. Load-induced gait changes were completely rescued by carprofen in mice loaded only once, whilst those in mice loaded repetitively persisted.

CONCLUSION: Our findings reveal specific and reproducible, compensatory changes in contralateral, non-loaded limb gait induced by any joint loading which produces focal articular lesions, and modified ipsilateral front limb use only when progressing lesions are induced by repetitive loading. We find that pain relief completely alleviates all gait modifications associated with stable lesions induced by single loading, but not those induced by repetitive loading. Differing responses in mice with stable and progressive articular cartilage load-induced lesions suggests that gait behaviour in a mechanical loading model of OA may predict joint degeneration.

P004

VISUALISATION OF THE APICAL SURFACE OF ACTIVATED OSTEOCLASTS

KA Szewczyk*[1], K Fuller[1], RF Moss[2], TJ Chambers[1]

[1]Department of Cellular Pathology, St George's Hospital Medical School,London,UK; [2]Department of Basic Medical Sciences, St George's Hospital Medical School,London,UK

Bone resorption occurs at the substrate-apposed, apical surface of osteoclasts, a surface that has not been accessible to direct visualisation. We have recently shown that it is the high affinity of bone mineral for vitronectin-receptor ligands that endows bone with the ability to activate osteoclasts. Consistent with this, osteoclasts secrete acid hydrolases and develop a ruffled border, as they do on bone, when they are incubated on vitronectin-coated plastic. Therefore, osteoclasts can be activated for resorption by substrates other than bone.

We have developed a novel approach to inspect the substrate-apposed surface of cells. To do this, cells are sedimented onto glass coverslips that have previously been coated with nail varnish. After incubation on such coverslips, the discs of nail varnish, with attached cells, are inverted onto a glass slide, and the nail varnish is removed with acetone. This exposes the underside of the cells, which can be inspected in the scanning electron microscope.

To analyse the substrate-apposed surface of activated osteoclasts, the nail varnish was coated with either vitronectin, or neonatal calf serum as a source of vitronectin. After incubation, the underside of osteoclasts showed a striking appearance. The whole undersurface was closely applied to the substrate, as it is on bone, in most osteoclasts. The central region was sometimes covered by fine, finger-like processes, often flattened against the substrate. More commonly the processes formed islands, or rings at the periphery of the central area. We also noted distinctive sucker-like structures of different sizes and shapes in almost all cells. Some of the central regions showed compartmentalisation, with compartmental walls similar in appearance to the stalks of 'suckers'. In some ruffled border regions we saw orifices, perhaps reflecting endocytic or exocytic processes.

The circumferential region appeared flat and featureless. However, if osmium tetroxide was omitted from fixation, rings, crescents and ridges of nodular protrusions, corresponding in position with podosomes, were observed circumferentially. The surface between and adjacent to podosomes, overlying the peri-podosomal cytoskeletal network, was extensively pitted.

This approach provides an exciting opportunity to characterise the morphological correlates of the resorptive process in osteoclasts.

P005

A TISSUE ENGINEERING APPROACH WITH TANTALUM TRABECULAR METAL TO ENHANCE BONE-IMPLANT INTEGRATION

JO Smith*[1], BG Sengers[2], A Aarvold[1], ER Tayton[1], DG Dunlop[1], ROC Oreffo[1]

[1]Bone and Joint Research Group, University of Southampton, UK;[2]School of Engineering Sciences, University of Southampton, UK

The osteo-regenerative properties of allograft have recently been enhanced by addition of autogenous skeletal stem cells to treat orthopaedic conditions characterised by lost bone stock. There are however, multiple disadvantages to allograft, including cost, availability, consistency and potential for disease transmission, and trabecular tantalum represents a potential alternative. Tantalum is already in widespread orthopaedic use, although in applications where there is poor initial implant stability, or when tantalum is used in conjunction with bone grafting, loading may need to be limited until sound integration has occurred. Development of enhanced bone-implant integration strategies will improve patient outcomes, extending the clinical applications of tantalum as a substitute for allograft.

The aim of this study was to examine the osteoconductive potential of trabecular tantalum in comparison to human allograft to determine its potential as an alternative to allograft.

Human bone marrow stromal cells (500,000 cells per ml) were cultured on blocks of trabecular tantalum or allograft for 28 days in basal and osteogenic media. Molecular profiling, confocal and scanning electron microscopy, as well as live-dead staining and biochemical assays were used to characterise cell adherence, proliferation and phenotype.

Cells displayed extensive adherence and proliferation throughout trabecular tantalum evidenced by CellTracker immunocytochemistry and SEM. Tantalum-cell constructs cultured in osteogenic conditions displayed extensive matrix production. Electron microscopy confirmed significant cellular growth through the tantalum to a depth of 5mm. In contrast to cells cultured with allograft in both basal and osteogenic conditions, cell proliferation assays showed significantly higher activity with tantalum than with allograft ($P<0.01$). Alkaline phosphatase (ALP) assay and molecular profiling confirmed no significant difference in expression of ALP, Runx-2, Col-1 and Sox-9 between cells cultured on tantalum and allograft.

These studies demonstrate the ability of trabecular tantalum to support skeletal cell growth and osteogenic differentiation comparable to allograft. Trabecular tantalum represents a good alternative to allograft for tissue engineering osteo-regenerative strategies in the context of lost bone stock. Such clinical scenarios will become increasingly common given the ageing demographic, the projected rates of revision arthroplasty requiring bone stock replacement and the limitations of allograft. Further mechanical testing and in vivo studies are on-going.

P006

HAPLOINSUFFICIENCY OF BAG-1 AFFECTS CARTILAGE DEVELOPMENT AND OSTEOGENIC DIFFERENTIATION IN VITRO

RS Tare*[1], PA Townsend[2], GK Packham[3], ROC Oreffo[1]

[1]Bone and Joint Research Group;[2]Human Genetics;[3]Cancer Sciences, University of Southampton, Southampton SO16 6YD, UK

The Bcl-2-associated athanogene-1 (Bag-1) co-chaperone is expressed by chondrocytes and osteoblasts, and functions as a transcriptional regulator mediating chondrocyte differentiation and apoptosis in vitro (1). By utilising cell populations from a mouse model of Bag-1 haploinsufficiency (i.e. one Bag-1 null allele and one normal Bag-1 allele), this study aims to define effects of Bag-1 heterozygosity on formation of cartilage templates and osteoblast development.

Micromass cultures of E11.5 Bag-1+/+ and Bag-1+/- mouse embryonic fibroblasts (MEFs), differentiated in presence of BMP-2 (100 ng/ml), ascorbate and beta-glycerophosphate, were utilised to model in vivo developmental stages in the formation of cartilaginous primordia. Cultures were analysed at days 7, 14, 21, 28, 35 and 42 for expression of Bag-1 and development of cartilage nodules. Bone marrow cells (BMCs) from 14-week Bag-1+/+ and Bag-1+/- mice were cultured for 21 days in media supplemented with either 10 nM Dexamethasone, 25 nM Calcitriol or 100 ng/ml BMP-2, to analyse their osteogenic response.

During the initial 14-day period, significantly high Bag-1 expression was observed in micromass cultures of Bag-1+/+ MEFs compared to Bag-1+/- MEFs. The 14-day period of micromass culture is comparable to in vivo murine embryonic limb development at E10.5 and E11.5, associated with robust Bag-1 expression and formation of vital mesenchymal condensations prefiguring future skeletal elements. Significantly, days 7 and 14 of Bag-1+/+ MEF micromass cultures were characterised by the presence of cellular condensations and mineralised cartilaginous nodules were observed by day 42 of culture. Bag-1 haploinsufficiency compromised the ability of Bag-1+/- MEFs to undergo condensations and organise into defined cartilaginous nodules by day 42 of micromass culture. Moreover, morphologically distinct hypertrophic chondrocytes (characterised by increase in cell volume and hence, enlarged cell size) in lacunae were absent in day 42 Bag-1+/- micromass cultures. Bag-1 heterozygosity also compromised the ability of BMCs to undergo robust osteogenic differentiation, as demonstrated by a decreased osteogenic response of Bag-1+/- BMCs compared to Bag-1+/+ BMCs in presence of the supplemented osteogenic factors.

Our results illustrate a role for Bag-1 in the critical phases of skeletal development, namely formation of the cartilage templates of endochondral bones and osteoblast differentiation.

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P007

CHONDROGIDE VERSUS PERIOSTEUM: A HISTOLOGICAL ANALYSIS

HS McCarthy*[1], S Roberts[1]

[1]Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Trust and ISTM (Keele University), Oswestry, Shropshire, UK

Autologous chondrocyte implantation (ACI) has been used for many years for the treatment of symptomatic defects in articular joints, predominantly the knee. Traditionally, cells were implanted behind a periosteal membrane, but in more recent times Chondrogide, a membrane consisting of porcine collagens I and III, has been used. There have been trials comparing the clinical outcome of these two groups of patients; in this study we compare the histological outcome using the two different patch types.

In a study of 100 patients having received ACI treatment of cartilage defects in the knee, 41 received Chondrogide (ACI-C) and 59 received periosteum (ACI-P). All of these patients had a post-operative biopsy taken at a mean of 16.9±9.2 months and 20.8±23.2 months for ACI-C and ACI-P respectively for histology using the ICRS II scoring system. Lysholm scores, a measure of knee function, were obtained pre- and post-operatively at the time of biopsy and statistical differences tested for via a Mann-Whitney U-test.

The mean age of the two groups at treatment was 37±8 and 35±10 years, the size of defect treated was 6.1±5.4 and 4.4±2.7 cm2 and the biopsy follow-up time was 50.6±22.2 and 81.2±34.8 months for ACI-C and ACI-P patients respectively. Both groups exhibited a significant improvement in Lysholm score from pre-operative to the time of biopsy (14.3±25.7; n=100), although there was no significant difference in improvement in Lysholm score between the two patch types. There was no significant difference between the histology score of the two groups, nor was the score found to correlate with the Lysholm score at that time. The individual components of the ICRS II score did not differ significantly with patch type (even for the surface architecture) apart from cellular morphology which was 6.5±3 and 8.2±1.6 for ACI-C and ACI-P respectively.

The histological quality of repair tissue formed with ACI-C differed little from that seen with ACI-P, despite the former group being biopsied ~4 months sooner after treatment and being used to treat defects which were 39% larger. Hence Chondrogide appears just as suitable as periosteum for use as a patch in the procedure of ACI.

P008

TEMPORAL ANALYSIS OF EMBRYONIC BONE DEVELOPMENT USING AN INNOVATIVE ORGANOTYPIC BONE CULTURE MODEL - APPLICATION OF MICRO-COMPUTED TOMOGRAPHY

JM Kanczler*, EL Smith, CA Roberts, ROC Oreffo

Bone & Joint Research Group, University of Southampton, Southampton, UK

Significant challenges exist for constructing complex tissues, such as bone, that can only be informed by a thorough understanding of the developing tissue environment. Understanding skeletal development of embryonic bone will offer new paradigms for bone augmentation. The organ culture of bone allows for skeletal cells to remain within their extracellular matrix structure while the external environment can be manipulated. The present study has investigated the development of embryonic bone and the effects of different culture conditions on chick femora development in a novel three-dimensional organotypic culture system using micro-computed tomography (microCT).

The length and structure of isolated embryonic chick femurs from E10-E17 were evaluated using microCT. Additionally, femurs were isolated at E10-E13 and placed in organotypic cultures for 10 days in foetal calf serum free culture medium (basal, chondrogenic or osteogenic conditions). Organotypic femurs were analyzed by microCT and assessed histologically for proteoglycans (alcian blue) and collagen (Sirius red) production.

In the developing embryonic chick, femur length (mm) increased from E10=4.9±0.5 to E17=14.2±0.8. microCT analysis (10micron resolution) demonstrated a 152-fold increase in Bone Volume/Total Volume (BV/TV); a 14-fold increase in Trabecular Thickness (TbTh(mm3); a 296-fold increase in Trabecular Number (TbNo(mm)) and a 280-fold decrease in Trabecular Spacing (TbSp (mm)). The embryonic chick femurs-E11 group demonstrated the most significant changes in microCT bone structural elements over the 10 day organotypic culture either in basal, chondrogenic or osteogenic conditions.

E11 isolated femurs, organotypically cultured for 10 days, increased their length compared to control femurs by 3.1mm (Basal); 2.0mm (Chondrogenic) and 2.4mm (Osteogenic) respectively. This was reflected in significant increases (***$P<0.001$) in microCT bone parameters; (BV/TV), TbTh(mm3), TbNo(mm) and decreased TbSp(mm) in the organotypic culture groups: (BV/TV): Control-E11=0.002±0.001; Basal (d10)=0.009±0.003; Chondrogenic (d10)=0.008±0; Osteogenic (d10)=0.014±0.002; Significant differences were observed in microCT bone parameters (***$P<0.001$) in the osteogenic organotypic culture

groups compared to basal and chondrogenic cultures. However, TbTh(mm3) remained the same across the culture groups.
The current studies demonstrate the efficacy of microCT to interrogate skeletal development. Furthermore, these studies demonstrate the ability to manipulate the developmental window. We believe understanding skeletal developmental biology will underpin and inform the skeletal regenerative process.

P009
HOW DOES VERTEBROPLASTY AFFECT ADJACENT VERTEBRAE?
J Luo*[1], DJ Annesley-Williams[2], MA Adams[1] and P Dolan[1]
[1]Centre for Comparative and Clinical Anatomy, University of Bristol, Bristol, UK;[2]Queen's Medical Centre, Nottingham, UK
Fracture of an osteoporotic vertebral body reduces vertebral stiffness and decompresses the nucleus in the adjacent intervertebral disc. This leads to high compressive stresses acting on the annulus and neural arch. Altered load-sharing at the fractured level may influence loading of neighbouring vertebrae, increasing the risk of a fracture 'cascade'. Vertebroplasty has been shown to normalise load-bearing by fractured vertebrae but it may increase the risk of adjacent level fracture. The aim of this study was to determine the effects of fracture and subsequent vertebroplasty on the loading of neighbouring (non-augmented) vertebrae.
Fourteen pairs of three-vertebra cadaver spine specimens (67-92 yr) were loaded to induce fracture. One of each pair underwent vertebroplasty with PMMA, the other with a resin (Cortoss). Specimens were then creep loaded at 1.0kN for 1hr. In 17 specimens where the upper or lower vertebra fractured, compressive stress distributions were measured in the disc between adjacent non-fractured vertebrae by pulling a pressure transducer through the disc whilst under 1.0kN load. These 'stress profiles' were obtained at each stage of the experiment (in flexion and extension) in order to quantify intradiscal pressure (IDP), the size of stress concentrations in the posterior annulus (SP) and compressive load-bearing by anterior (FA) and posterior (FP) halves of the vertebral body and by the neural arch (FN).
No differences were found between Cortoss and PMMA so all data were pooled. Following fracture, IDP fell by 26% in extension (P=0.004) and SP increased by more than 200% in flexion (P=0.01). FA decreased from 55% to 36% of the applied load in flexion (P=0.002) and from 36% to 27% in extension (P=0.002). FN increased from 17% to 31% in flexion (P=0.006) and from 22% to 37% in extension (P=0.008). Vertebroplasty reduced stress concentrations in the disc and restored load-bearing towards pre-fracture values.
Vertebral fracture transfers compressive load from the anterior vertebral body to the posterior vertebral body and neural arch of adjacent (non-fractured) vertebrae. Vertebroplasty largely restores normal load-sharing at both the augmented and adjacent levels and in doing so may help reduce the risk of a spinal fracture cascade.

P010
MODULATION OF ENDOGENOUS FPP SYNTHASE CAUSES BISPHOSPHONATE RESISTANCE IN CULTURED CELLS
S Das*, JC Crockett and MJ Rogers
University of Aberdeen, UK
Nitrogen containing bisphosphonates (N-BPs), such as zoledronic acid (ZOL), are potent inhibitors of osteoclast function and act by preventing the post-translational prenylation of small GTPase signalling proteins such as Rap1A. Isoprenoid lipids, required for prenylation, are generated via the mevalonate pathway which is also responsible for cholesterol biosynthesis. Farnesyl diphosphate (FPP) synthase, a key enzyme in this pathway, is the molecular target for N-BPs and it is likely that modulation of FPP synthase expression may affect flux through the pathway and therefore levels of prenylated proteins within cells. In cell culture, N-BPs can affect many cell types but there is variability between different cells in the sensitivity to N-BPs observed. In addition, not all patients respond in the same way to N-BP therapy and it has been reported that some patients develop resistance. It is not known if upregulation of FPP synthase may contribute to the variability in sensitivity to N-BPs or, indeed to this resistance. Since sterol deficiency induces SRE-mediated upregulation of FPP synthase we postulate that upregulation of endogenous FPP synthase in cultured cells in the presence of lipoprotein depleted serum (LDS) would reduce the effectiveness of N-BP treatment.
When HeLa cells were treated with 2 micromolar or more ZOL for 24 hours in 10% foetal calf serum, unprenylated Rap1A could be detected by western blot analysis and the levels of FPP synthase mRNA and protein remained unchanged, which was probably as a result of the presence of exogenous sterols in the culture medium. However, in the presence of 10% LDS for 48 hours, both the FPP synthase mRNA and protein levels increased by at least 3 fold and unprenylated Rap1A was not detected following treatment with up to 10 micromolar ZOL indicating resistance to ZOL due to upregulation of FPP synthase. Therefore, sterol mediated upregulation of FPP synthase is a model for observing N-BP resistance, but it remains to be determined whether such regulation could occur in different cell types, including osteoclasts, in vivo and whether this could explain some of the variability in response to N-BP treatment in the clinic.

P011
VOLUMETRIC WEAR ASSESSMENT OF FAILED METAL-ON-METAL HIP RESURFACING PROSTHESES
JK Lord[1], DJ Langton[1], AVF Nargol[2], TJ Joyce*[1]
[1]School of Mechanical & Systems Engineering, Newcastle University, UK; [2]Joint Replacement Unit, University Hospital of North Tees, Stockton, UK
Wear debris induced osteolysis is a recognized complication in conventional metal-on-polyethylene hip arthroplasty. One method of achieving wear reduction is through the use of metal-on-metal articulations. One of the latest manifestations of this biomaterial combination is in designs of hip resurfacing which are aimed at younger, more active patients. But, do these metal-on-metal hip resurfacings show low wear when implanted into patients?
Using a Mitutoyo Legex 322 co-ordinate measuring machine (scanning accuracy less than 1 micron) and a bespoke computer program, volumetric wear measurements for retrieved Articular Surface Replacements (ASR, DePuy) metal-on-metal hip resurfacings were undertaken. Measurements were validated against gravimetric calculations for volumetric wear using a sample femoral head that was artificially worn in vitro. At 5mm3, 10mm3, and 15mm3 of material removal, the method was shown to be accurate to within 0.5mm3.
Thirty-two femoral heads and twenty-two acetabular cups were measured. Acetabular cups exhibited mean volumetric wear of 29.00mm3 (range 1.35 - 109.72mm3) and a wear rate of 11.02mm3/year (range 0.30 - 63.59mm3/year). Femoral heads exhibited mean wear of 22.41mm3 (range 0.72 - 134.22mm3) and a wear rate of 8.72mm3/year (range 0.21 - 31.91mm3/year). In the 22 cases where both head and cup from the same prosthesis were available, mean total wear rates of 21.66mm3/year (range 0.51 - 95.50mm3/year) were observed.
Revision was necessitated by one of five effects; early femoral neck fracture (4 heads), avascular necrosis (AVN) (2 heads, 1 cup), infection (1 head, 1 cup), adverse reaction to metal debris (ARMD) (19 heads, 18 cups) or ARMD fracture (6 heads, 2 cups). Mean paired wear rates for the AVN and infection retrievals were 0.51mm3/year and 3.98mm3/year respectively. In vitro tests typically offer wear rates for metal-on-metal devices in the region of 2-4mm3.
Mean paired wear rates for ARMD and ARMD fracture were 17.64mm3/year and 68.5mm3/year respectively, significantly greater than those expected from in vitro tests. In the 4 cases of early fracture, only the heads were revised so a combined wear rate calculation was not possible. The heads exhibited mean wear rate of 8.26mm3/year. These high wear rates are of concern.

P012
THE EFFECT OF HYPERBARIC OXYGEN THERAPY ON OSTEOCLAST FORMATION AND BONE RESORPTION
HW AL-Hadi*[1],SW Fox[1],GR Smerdon[2]
[1]School of Biomedical and Biological Science, University of Plymouth, UK;[2]The Diving Diseases Research Centre, Plymouth, UK
Hyperbaric oxygen therapy (HBO) is the breathing of pure oxygen in a sealed chamber at greater than normal atmospheric pressure. HBO is employed as an adjunctive therapy in a number of skeletal disorders such radio and bisphosphonate induced osteonecrosis of the jaw and chronic osteomyelitis. These disorders are associated with aberrant remodelling and excessive levels of resorption leading to increased fracture risk. The purpose of the present study was to evaluate the effect of HBO, pressure and hyperoxia on RANKL-induced osteoclast formation in RAW 264.7 cells and human peripheral blood monocytes (PBMC).
Daily, HBO (2.4 ATA, 97% O_2, 90 minutes per day) or hyperoxia (1 ATM, 95% O_2, 90 minutes per day) treatments significantly decreased the number of RANKL-induced TRAP positive mononuclear and multinuclear osteoclasts forming in RAW and PBMC cultures ($p < 0.05$). Similarly, HBO and hyperoxia significantly reduced bone resorption as assessed by the bone slice assay. Furthermore, pressure alone (2.4 ATA, 8.8% O_2, 90 minutes per day) also significantly suppressed osteoclast differentiation and bone resorption to a similar extent as HBO and hyperoxia. Quantitative PCR analysis of key regulators of osteoclast differentiation (RANK) and multinuclearity (DC-STAMP) indicated that HBO, hyperoxia and pressure all significantly suppressed RANK and DC-STAMP mRNA expression. Interestingly, intermittent HBO, hyperoxia and pressure (daily, 90 minutes per day) also significantly reduced RANKL-induced osteoclast formation in hypoxic conditions (2% O_2, 22.5 hours per day).
This data suggests that HBO, elevated O_2 and pressure suppress osteoclast differentiation and bone resorption in mouse and human monocytes. Furthermore the data indicates that elevated pressure and O_2 also directly inhibit osteoclast formation in hypoxic conditions a hallmark of many skeletal disorders. This provides evidence supporting the use of HBO as an adjunctive therapy to prevent bone loss in a range of skeletal disorders associated with low oxygen partial pressure.

P013
STUDY AND DESIGN OF A PROTOCOL FOR AUTOLOGOUS DRAIN USE FOR TOTAL KNEE REPLACEMENTS AT THE ROYAL DEVON AND EXTER HOSPITAL (RD&E)
WJ White*[1], J Harvey[2], AD Toms[3]
[1]Royal Devon and Exeter Hospital, UK

Allogeneic blood transfusion is associated with many complications and significant cost. The RD&E has looked at the use of autologous drains after our study of 100 cases showed an improved post-operative haemoglobin and reduced length of stay. There is a need to identify those patients of increased need for an autologous drain, in order to decrease the frequency of allogeneic transfusion. In 2007 a protocol was drawn up using information from our study of 191 cases which showed an average haemoglobin drop post-operatively of 3.05g/dl and average intra-operative blood loss of 285 ml. This protocol gives the surgeon triggers for autologous drain use; preoperative haemoglobin of <13g/dl, intra-operative blood loss of >400ml, tourniquet use, patient weight <50kg and patients refusing donated blood.
In 2007-08, 65% of a further 275 cases analysed met the triggers for use of an autologous system. The remaining patients received low vacuum drains. Of the 275 patients, only 2 (<1%) of those who did not fulfil the criteria for an autologous drain required allogeneic blood, compared with 43 patients (24%) of those deemed high risk of transfusion, and assigned autologous drains. The protocol was therefore deemed to be successful in identifying those patients who required additional support and expenditure to minimise allogeneic blood transfusion.
Analysis of this data led to recommended changes to the protocol in order to maximise the efficiency of the autologous drain use. In 2010 a further patient cohort studied showed a reduction in allogeneic blood transfusion to <10% of those receiving autologous drains, and an increase to 5% of those with low vacuum drains.
Due to the increased cost of autologous drains (£68) compared with the low vacuum systems (£32), and the cost of allogeneic units at £141, the expenditure per patient was calculated and shown to fall from £92 in 2007 to £78 in the 2010.
In conclusion, this protocol allows the clinician to appropriately target the use of the more expensive autologous drains to those of increased risk of transfusion. This protocol helps to minimise unnecessary allogeneic blood transfusion risks, and this has been shown to be more cost effective.

P014
COMBINATION THERAPY BISPHOSPHONATES AND rhGH FOR CHILDREN WITH OSTEOGENESIS IMPERFECTA
C Galesanu*[1], N Lisnic[1], A Florescu[1], AI Loghin[1], V Zaharia[1], L Apostu[1]
[1]Department of Endocrinology, University of Medicine and Pharmacy 'Gr.T.Popa' Iasi. Romania
Introduction: Osteogenesis Imperfecta (OI) is a group of genetic disease with a wide spectrum of severity, ranging from very mild bone fragility to lethal forms. Patients with OI type I A have a mild phenotype with normal or near-normal height. The addition of recombinant human growth hormone (rhGH) to ongoing treatment with bisphosphonates can increase measures of BMD and growth.
Objectives: We studied growth rate, bone density and bone metabolism in two patients affected by type IA O.I. and growth delay.
Material and methods: Eight children (six girls and two boys) with OI type IA were treated with Risedronate. Among them two girls with mean age 6.5 years presented growth delay. These girls were treated with rhGH at a dose 0.2 mg/kg/week for two years. Auxologic data were measured every 3 months, bone age was determined at the start and at every six months. At every 3 months IGF 1, osteocalcin, alkalinphosphatase, calcium and phosphorus levels and urinary hydroxyproline and calcium levels were determined. Bone mineral density (BMD) measurements were made at the start and repeated at 12 and 24 months at the lumbar spine and whole body by DXA.
Results: After 24 months, linear growth velocity increased significantly: from 4.5 cm/yr. to 8 cm/yr. in the first year and to 8.5 cm/yr. in the second year. DXA-BMD at lumbar spine increased significantly from 0.359 g/cm2 to 0.464 g/ cm2 (+29 %) in the first year and at 0.505 g/cm2 (+9%) in the second one. Whole body - bone density improved +36% in the first year and 4.4% after second year. Serum osteocalcin levels increased significantly after rhGH treatment from 1.9±0.8 nmol/L at 3.9±0.9 nmol/L after the second year.
Conclusion: rhGH treatment addition at bisphosphonates in O.I. type I, increases significantly the rate of linear growth velocity. The bone turnover increases also and the bone mineral density in lumbar spine and whole body increases; the fracture risk does not increase. More children should be treated with rhGH to see the long term benefits of this treatment in final adult height and quality of OI bone.

P015
PHOSPHATASE AND TENSIN HOMOLOGUE, DELETED ON CHROMOSOME TEN (PTEN): A REVIEW ARTICLE
IH Abdulkareem*
Weston General Hospital, UK
Phosphatase and tensin homologue deleted on chromosome ten (PTEN), is a tumor suppressor gene discovered in 1997 (Li et al). It is absent or abnormal in many human cancers such as glioblastoma of the brain/spinal cord, as well as endometrial, prostate, bladder, adrenals, thyroid, breast, soft tissue, mesenchymal, and colon cancers, among others. These are associated with loss of heterozygosity (LOH) for the PTEN gene on chromosome 10q23. PTEN is a 403 amino acid protein with lipid phosphatase activity for phosphatidylinositol 3, 4, 5-triphosphate (PIP3). It down-regulates the PI3/Akt signaling pathway by dephosphorylating PIP3, and leads to inhibition of growth factor signal transduction, and prevention of growth promoting and anti-apoptotic effects of Akt kinase. The overall effect is regulation of cell-cycle progression, translation, apoptosis, cell size, growth, proliferation, and migration. Previous studies suggested that various drugs (NSAIDs), chemicals, and food supplements can up-regulate the PTEN mRNA and protein expression in different cell lines, implying that they may therefore be used in future for the prevention and/or treatment of some human cancers associated with LOH of PTEN.
We carried out a study to look at expression and sub-cellular localisation of PTEN protein, as well as the effect(s) of indomethacin on expression of in human endometrial cancer (Hec 1B) cell line, which is known to express significant amounts of the wild-type PTEN. The protein expressions and localisations were studied using immunocytochemistry and fluorescent microscopy.
The results revealed that the cultured Hec 1B cells expressed PTEN protein, most of which was localized in the nucleus, with minimal cytoplasmic expression. Increased PTEN expression was also observed following treatment with indomethacin. This agrees with previous literature and promises to be an exciting prospect in the future screening and treatment of many human cancers. However, further investigations with Western blotting or PCR are required, to confirm or refute these observations.

P016
ARE FLOWTRON BOOTS JUSTIFIED AS MEANS OF MECHANICAL PROPHYLAXIS FOR DVT FOLLOWING MAJOR ARTHROPLASTIES?
IH Abdulkareem*
Weston General Hospital, UK
Several types of pneumatic compression devices have been described and recommended for prophylaxis of DVTs/PEs. These are Graduated Compression Stockings, and Intermittent Pneumatic Compressions, whch decrease DVT incidence to 0.8% and 1.3% respectively.They include foot pumps (A-V Impulse system and PlexiPulse Foot), a foot-calf pump (PlexiPulse Foot-Calf), a calf pump (VenaFlow system), and calf-thigh pumps (SCD system, Flowtron DVT and Jobst Athrombotic Pump). All, especially those with calf compression, increase venous volume and capacity. They also decrease venous stasis and improve emptying, but may cause fibrinolysis. Also, devices with pulsatile pumping are better than those causing slow rise in venous return. Following major arthroplasties, prophylaxis for DVT/PEs are essential in order to minimise risk.
Our study included 54 patients who had hip (66%) and knee (34%) arthroplasties, with average age of 72.6 years, and predominantly female cohort (>60%). Only 53% had TEDS (Thrombo-Embolic Deterrent Stockings), but all had chemical prophylaxis (using Pradaxa, Clexane or Aspirin), and used Flowtron boots, with variable durations and times of application in relation to surgery. Only 57% had Flowtron applied from recovery, and 43% applied on the ward. The frequency of Flowtron discontinuation was up to eight times in 24 hours, in some patients, with an average duration of stops, of 83 minutes/24 hours. The most common reasons for removing or disconnecting boots were toilet, physiotherapy and x-rays. This was not necessary as the machine is portable and can be taken along with the patient. Two patients had positive CTPA for PEs, based on clinical suspicion. This gives a rate of 3.77%, which is almost double the 1-2% commonly quoted in the literature, and may suggest failure of the three preventive methods used (two types of mechanical, as well as various forms of chemical prophylaxis). Therefore, it is difficult to know the responsible factor for the apparently high PE rate in our cohort. It is likely that inadequate compliance with recommended guidelines on use of Flowtron as mechanical prophylaxis, may have contributed to this. Therefore, there is need for staff and patients to be educated on values of mechanical methods of DVT prevention. Flowtron should be used continuously from admission for up-to 72 hours, or until the patient becomes mobile.

P017
DIAGNOSTIC ACCURACY OF MRI AND MRA FOR TRIANGULAR FIBROCARTILAGINOUS COMPLEX INJURIES
TO Smith*[1], B Drew[2], AP Toms[3], C Jerosch-Herold[1], AJ Chojnowski[4]
[1]Faculty of Medicine and Health Sciences, University of East Anglia, UK; [2]Physiotherapy Department, West Suffolk Hospital, UK; [3]Department of Radiology, Norfolk and Norwich University Hospital, UK; [4]Institute of Orthopaedics, Norfolk and Norwich University Hospital, UK
Background and Objectives: Triangular fibrocartilaginous complex (TFCC) tears are common sources of ulna sided wrist pain and resultant functional disability. Diagnosis is based on history, clinical examination and radiological evidence of a TFCC central perforation or radial/ulna tear. The purpose of this study is therefore to evaluate the diagnostic accuracy of Magnetic Resonance Imaging (MRI) and Magnetic Resonance Arthrography (MRA) in the detection of TFCC injury in the adult population.
Methods: Published and unpublished literature databases were systematically review independently by two researchers. Two-by-two tables were constructed to calculate the sensitivity and specificity of MRI or MRA investigations against arthroscopic outcomes. Pooled sensitivity and specificity values and summary Receiver Operating Characteristic curve (sROC) evaluations were performed.

Methodological quality of each study was assessed using the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) tool.
Results: Twenty one studies were eligible, including 910 wrists. On meta-analysis, MRA was superior to MRI in the investigation of complete TFCC tears with a pooled sensitivity of 0.75 (95% Confidence Interval (CI): 0.70, 0.79) and specificity of 0.81 (95% CI: 0.76, 0.86), compared to MRAs 0.84 (95% CI: 0.79, 0.89), and 0.95 (95% CI: 0.92, 0.98) respectively. MRA and MRI performed at greater field strengths reported greater sensitivity and specificity findings. For 3.0 Tesla (T) MRI, the meta-analysis indicated a sensitivity of 0.86 (95% CI: 0.65, 0.97), and specificity of 1.00 (0.87, 1.00). In comparison, the pooled sensitivity for the 1.5T MRI assessment was 0.70 (95% CI: 0.64, 0.75) and specificity of 0.79 (95% CI: 0.72, 0.85). This trend was repeated for MRA where 3.0T MRA exhibited a sensitivity was 1.00 (95% CI: 0.79, 1.00) and specificity of 1.00 (95% CI: 0.82, 1.00), whilst pooled analysis 1.5T MRA demonstrated a sensitivity of 0.83 (95% CI: 0.78, 0.89) and specificity of 0.95 (95% CI: 0.91, 0.98). There was insufficient data to assess the diagnostic test accuracy of partial TFCC lesions.
Conclusions: Given its acceptable diagnostic test accuracy, it is recommended that in cases where there are questions over the diagnosis and subsequent management of patients with ulna wrist pain, a MRA should be undertaken rather than MRI.

P018
CATCH BEFORE A FALL - BUILDING AN IPAD APPLICATION FOR OSTEOPOROSIS RISK ASSESSMENT
SAW Grange*[1], GB Wills[2], L Gilbert[2], M Santer[2], A Recio[2], M Kanani[2], P Zhang[2], P Smitham[1]
[1]Institute of Orthopaedics and Musculoskeletal Science, Royal National Orthopaedic Hospital, Stanmore, UK; [2]Electronics and Computer Science, University of Southampton, Southampton, UK
Background and objectives: The prevention of osteoporotic fractures is a global problem. Key to this strategy is efficient identification of 'at risk' patients in order to address the osteoporosis pandemic, including the identification of previously sustained fractures. GP practices are now integrating touch screens as a method of registering patients' attendance for an appointment, so all ages of patients are becoming familiar with this channel of communication. Our touch screen patient administered questionnaire system intends to provide an effective solution.
Methods: The Virtual Research Integration Collaboration (VRIC) framework supports the integration of basic science and clinical research. It enables the management of research lifecycles by integrating scientific approaches with everyday work practice in a virtual research environment (VRE). 'Catch Before a Fall' (CBaF) is a clinical research project using VRIC, using a dedicated interface, co-designed by orthopaedic surgeons and basic scientists, adapted for sensory and IT impaired subjects to capture such information, since approximately 75% of registered over 65 year olds visit their GP each year.
Results: Established in test sites across the UK, Data analysis is conducted via the VRIC 'on-line' portal. The conclusion of the research process is followed up within that tool. Using the validated osteoporosis risk questionnaire augmented by self reporting of height loss to identify missed vertebral fractures, we calculate the patients' risk factor of developing osteoporosis and of having an osteoporosis related fracture within the next 10 years. Patients' data are collected through CBaF (figure 1) and stored in data structures matching the VRIC architecture for automatic importing via a dedicated script and offering direct clinical service provider feedback.
Conclusion: Patients recollect a previous fracture including other risk factors, so we are automating the secure data collection process to improve efficiency and save resources. We should see a 'win' for the patient who will receive better informed care. CBaF supports the practice who will streamline their pathway for effective osteoporosis management. The insight into personalised care management is a pathfinder, demonstrating improvement of services for our community, should reduce the greater silent population of osteoporosis sufferers worldwide, addressing the acute service burden 'at source'.

P019
EARLY SUBSIDENCE RATE OF COLLARLESS FEMORAL STEM
M Al-Najjim*[1], I Chambers[2]
Scunthorpe General Hospital, Scunthorpe, UK
To assess the early subsidence rate of the femoral stem for patients who had collarless Corail total hip replacement.
Consecutive data was collected retrospectively between August 2007 and December 2009 for patients who had collarless Corail total hip replacement. Radiographic assessment of the degree of subsidence, calcar resorption, stem angulation, canal fill ratio and loosening of the stem were measured. Post operative pain, dislocation and stem revision surgery were also evaluated.
48 patients were identified, providing 51 hips for the study. There were 22 male and 26 female. The mean age 64.2 years (range 38-77). Post-operative radiographs were taken at day 1, 6 weeks and one year post-operatively (range 10-18 months, mean 12.7).
Significant subsidence was defined as 3 or more millimetres, we identified two patients with subsidence between 3-5mm, one patient with 6mm and two patients with 10mm subsidence at one year post-operatively.
In the 5 patients with subsidence post-operatively, all significant subsidence occurred within the first 6 weeks.
Canal fill ratio was measured in all patients; in the non-subsided group the ratio was an average of 72% in the lower third of the stem and 84% in the middle third. In the subsided group 75% in the lower third of the stem and 81% in the middle third, which we felt was clinically insignificant.
There was no dislocation or revision for septic loosening. One patient with 10mm subsidence had liner revision for dissociation but the stem was well fixed and not revised.
All patients who had significant subsidence still had functioning implants with no pain, revisions for subsidence or features of loosening.
There were no radiological features predictive of subsidence in our patients.
Subsidence may be due to lack of compliance in some patients with partial weight bearing and increased Body Mass Index.

P020
USE OF HAND MADE ANTIBIOTIC LOADED CEMENT BEADS FOR ACUTE INFECTIONS IN TRAUMA SURGERY WHILST RETAINING THE FIXATION DEVICE
G Abbas*, H Versey, AN Murty
Wansbeck General Hospital, Woodhorn Lane, Ashington, Northumberland NE63 9JJ, UK
Antibiotic-loaded bone cement is widely used to treat bone and joint infections. Commercially available materials contain only a small dose of antibiotics that might not be adequate to eradicate deep musculoskeletal infections. Aim of this study was to assess the efficacy of using combination of vancomycin and gentamicin in hand made cement beads in treating acute infections in orthopaedic trauma surgery whilst retaining the fixation device.
After clinical, Haematological, biochemical, and microbiological assessment, deep infections were diagnosed in 16 patients with musculoskeletal trauma treated with open reduction and internal fixation between September 2007 and March 2010. There were 11 males and 5 females with mean age of 49.375 years (24 to 78).
A through debridement was carried out with excision of all the infected and dead tissue from the various infected fracture sites involving distal tibia, tibial plateau, ankle, femur, ulna, humerus and clavicle. Fixation implant was retained in all patients during surgical debridement expect one.
After the debridement, antibiotic loaded cement beads were prepared without using negative pressure by adding 2 grams of vancomycin and 4 grams of gentamicin powder to 40 g pack cement. The beads were then inserted into the infected fracture sites before wound closure.
Empirical systemic antibiotic therapy was administered according to the results of gram staining before obtaining the final microbiology report. The patients were reviewed clinically, radiologically and serologically at three, six and 12 months after surgery and then annually.
After a mean follow-up of 15.75 months (9-24), 15 patients were infection free with clinical and radiological union of their fractures with retention of fixation device.
We conclude that early and aggressive surgical treatment of acute deep infections in trauma surgery with high dose hand made antibiotic loaded cement beads can be effective and fixation device can be retained.

P021
WHAT IS THE COST OF TREATING SUPERFICIAL AND DEEP INFECTIONS IN TOTAL HIP AND KNEE REPLACEMENT SURGERY?
G Abbas*[1], A Sprowson[2], T Symes[3], MR Reed[4]
Wansbeck General Hospital, Woodhorn Lane, Ashington, Northumberland NE63 9JJ, UK
Joint infection is a major cause of morbidity, mortality and has a considerable economic burden for the health care service. The aim of this study was to quantify the real cost paid by the originating Primary Care Trust (PCT) to the hospital via payment by results (PbR) income for managing infections in hip and knee joint replacement surgery.
Twenty three consecutive cases of superficial and deep infection in total joint replacement surgery of knee and hip over a 15 month period were retrospectively analysed. We reviewed clinical and financial data to determine the resources needed and allocated. The financial data included the cost of all subsequent operations for both superficial and deep infections, which were assessed separately. The average cost for treating superficial infection in TKR was ú6,577 as compared to ú6,465 in THR. The average cost of treating deep infection was ú14,335 and ú17,509 for TKR and THR respectively. When the cost was assessed on a patient to patient basis, large discrepancies to the factor of four times were identified. The analysis illustrates the continuing problems with coding complex patients with multiple procedures, such as infected joint replacements. In this current cold economical climate, it is important that clinicians are actively involved in financial aspect of health provision and its ongoing problems.

P022
INNATE ARTHROSCOPIC SKILLS IN MEDICAL STUDENTS AND VARIATION IN LEARNING CURVES
A Alvand*, S Auplish, HS Gill, JL Rees

Oxford Orthopaedic Simulation & Education Centre, Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences
NIHR Biomedical Research Unit, University of Oxford, UK
Background: Technical skill is an essential domain of surgical competency. Arthroscopic surgery forms a particularly challenging subset of these skills. The innate ability to acquire these skills is not fully understood. The aim of this study was to investigate the innate arthroscopic skills and learning curve patterns of medical students - our future surgeons.
Methods: Two arthroscopic tasks (one shoulder and one knee) were set up in a bioskills laboratory to represent core skills required for arthroscopic training. Twenty medical students with no previous arthroscopic surgery experience were recruited and their performance assessed whilst undertaking each task on 30 occasions. The primary outcome variable was success or failure. Individuals were assessed as 'competent' if they stabilised their learning curve within 20 episodes. The secondary outcome measure was an objective assessment of technical dexterity using a validated Motion Analysis system (time taken to complete tasks, total path length of the subject's hands, and number of hand movements).
Results: There was variability in the performance of the students. Seven students in the shoulder task and four students in the knee task were unable to achieve competence. Motion analysis data demonstrated that students who achieved task competence had better objective technical dexterity and therefore better innate arthroscopic ability. For the shoulder task, these differences were statistically significant for 'path length' and 'hand movement' ($p<0.05$, Mann-Whitney U test). For the knee task, the differences were statistically significant for 'path length' ($P<0.05$, Mann-Whitney U test).
Conclusion: Variation in innate arthroscopic skill exists in our future surgeons with some individuals being unable to achieve competence at basic arthroscopic tasks despite sustained practice. It may be of great value to identify individuals who lack innate arthroscopic skills early in their career in order to provide them with focused training and relevant career guidance.

P023
THE IMPACT OF TRAINING ON THE ARTHROSCOPIC PERFORMANCE OF MEDICAL STUDENTS: A RANDOMISED STUDY
A Alvand*, S Auplish, HS Gill, JL Rees
Oxford Orthopaedic Simulation & Education Centre, Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences, NIHR Biomedical Research Unit, University of Oxford, UK
Background: The ability to learn arthroscopic surgery is an important aspect of modern day orthopaedic surgery. Knowing that variation in innate ability exists amongst medical students, the aim of this study was to investigate the effect of training on the arthroscopic surgical performance of our future orthopaedic surgeons (medical students).
Methods: Two arthroscopic tasks (one shoulder and one knee) were set up in a bioskills laboratory to represent core skills required for arthroscopic training. Thirty three medical students with no previous arthroscopic surgery experience were randomised to a 'Trained' (n=16) and 'Non-trained' (n=17) cohort. Both groups watched an instructional video. The Trained cohort also received specific training on the tasks prior to their first episode. Thirty episodes of each task were then undertaken. The primary outcome variable was success or failure. Individuals were assessed as 'competent' if they stabilised their learning curve within 20 episodes. The secondary outcome measure was an objective assessment of technical dexterity using a validated Motion Analysis system (time taken to complete tasks, total path length of the subject's hands, and number of hand movements).
Results: During the shoulder task, one subject in the Trained cohort failed to achieve competence compared with six subjects in the Non-trained cohort. During the knee task, two subjects in each cohort failed to achieve competence. Performance of the subjects in the Trained cohort during the shoulder task was significantly better ($p<0.05$, Chi-squared test). Based on the objective motion analysis parameters, the Trained cohort performed better than the Non-trained cohort for both tasks. This was statistically significant ($p<0.05$, Mann-Whitney U test) for the shoulder task.
Conclusion: As expected, specific training can improve the arthroscopic performance of novices. There were, however, individuals who could not achieve competency in basic arthroscopic tasks even with focused training. Such assessments might influence students' future career choices.

P024
COMBINED ANTERIOR CRUCIATE LIGAMENT REPAIR AND AUTOLOGOUS CHONDROCYTE IMPLANTATION
BS Dhinsa*[1], SZ Nawaz[1], KR Gallagher[1], R Carrington[1], TWR Briggs[1], JA Skinner[1], G Bentley[1]
Joint Reconstruction Unit, Royal National Orthopaedic Hospital, UK
Introduction: Autologous chondrocyte implantation (ACI) is contra-indicated in a joint rendered unstable by a ruptured anterior cruciate ligament (ACL). We present our experience of ACI repair with ACL reconstruction
Methods: Patients underwent arthroscopic examination and cartilage harvesting of the knee. A second operation was undertaken approximately six weeks later to repair the ruptured ACL with hamstring graft or Bone patella-Bone (BPB) and to implant the chondrocytes via formal arthrotomy. Three groups were assessed: Group 1: Simultaneous ACL Reconstruction and ACI; Group 2: Previous ACL Reconstruction with subsequent ACI repair; Group 3: Previously proven partial or complete ACL rupture, deemed stable and not treated with reconstruction with ACI procedure subsequently. Patients then underwent a graduated rehabilitation program and were reviewed using three functional measurements: Bentley functional scale, the modified Cincinnati rating system, and pain measured on a visual analogue scale. All patients also underwent formal clinical examination at review.
Results: Those who underwent simultaneous ACL Reconstruction and ACI had a 47% improvement in Bentley functional scale, 36% improvement in visual analogue score and 38% improvement in the modified Cincinnati rating system. This is in contrast to only a 15% improvement in the modified Cincinnati rating system, 30% improvement in Bentley functional scale, and 32% improvement in visual analogue score in patients who had ACI repair after previous ACL reconstruction. 68% of patients who had the procedures simultaneously rated their outcome as excellent/good and 27% felt it was a failure. In contrast 38% of patients rated their outcome as a failure if they had ACI repair without reconstruction of ACL rupture.
Conclusion: Symptomatic cartilage defects and ACL deficiency may co-exist in many patients and represent a treatment challenge. Our results suggest that a combined ACL and ACI repair is a viable option in this group of patients and should reduce the anaesthetic and operative risks of a two-stage repair. Patients with complete rupture of ACL despite being deemed stable performed poorly at review and our study suggests all complete ruptures regardless of stability should be treated with a reconstruction when performing an autologous chondrocyte implantation.

P025
NEONATAL KNEE ULTRASOUND: A NEW METHOD FOR ASSESSING DISTAL FEMUR MORPHOLOGY IN PERINATAL VITAMIN D INSUFFICIENCY
PA Mahon*[1], G Ntani[1], C Cooper[1,2,3], KM Godfrey[1,2], MK Javaid[3], NC Harvey[1]
[1]MRC Lifecourse Epidemiology Unit, (University of Southampton), UK; [2]NIHR Nutrition, Diet and Lifestyle Biomedical Research Unit, Southampton University Hospitals NHS Trust, Southampton General Hospital, Southampton, UK; [3]Oxford NIHR Musculoskeletal Biomedical Research Centre, University of Oxford, UK
We have previously demonstrated that lower maternal circulating 25(OH)-vitamin D concentrations in late pregnancy are associated with reduced offspring bone mass postnatally, and altered distal femoral morphology in utero, reminiscent of metaphyseal changes observed in postnatal rickets. We therefore aimed to develop a technique that would allow investigation of whether distal femoral morphology differed in offspring born to women who were supplemented with vitamin D or placebo in a randomised, double-blind, placebo-controlled trial (MAVIDOS: Maternal Vitamin D Osteoporosis Study).
Pregnant women recruited to the MAVIDOS study at their 11 week nuchal or dating scan are randomised to either 1000 IU cholecalciferol daily or matched placebo until delivery of the baby. Fetal measurements are taken using ultrasound at 11, 19 and 34 weeks gestation, with dual energy X-ray absorptiometry of the neonate at birth. From the 400 pregnant women recruited to date a sub-set of 142 neonates underwent 3D ultrasound assessment of the distal femoral metaphysis within 2 weeks after delivery. Measurements of epiphyseal volume (using VOCAL technique), central epiphyseal-metaphyseal distance (CEMD) and cross-sectional area (CSA) were taken in triplicate from stored images. Values were adjusted for gestational age and then assessed for reproducibility. The coefficients of variation (CVs) were 0.98% for linear CEMD, 0.83% for epiphyseal volume and 2.17% for traced CSA measurements. These results demonstrate good reproducibility. Additionally, the between-subject variability was assessed and showed a sizeable range of dimensions consistent with biological variation: between subject CVs for CEMD, epiphyseal volume and CSA were 28.7%, 49.4% and 17.3%, respectively.
These results suggest that the technique is reproducible and may yield meaningful biological data. Images on further participants will be acquired and at the end of the trial, comparisons will be made of measurements in babies of supplemented and unsupplemented mothers. Potentially, this will add valuable information regarding the role of maternal vitamin D status in offspring bone development. This non-invasive technique may also be utilised in longitudinal assessments during childhood follow-up studies, allowing the longer term evaluation of effects originating in utero.

P026
IS LENGTHENING OVER NAIL (LON) BETTER THAN INTRAMEDULLARY SKELETAL MEDULLARY DISTRACTION (ISKD) FOR FEMORAL LENGTHENING?
S Mahboubian, KTM Seah*, AT Fragomen, L Schacter, SR Rozbruch
Hospital for Special Surgery, New York, USA
Background: Lengthening over nail (LON) and the use of internal lengthening nails have been developed to minimize patients' time in a frame during femur lengthening. This study compares the outcomes of two techniques of femur lengthening, LON and Intramedullary Skeletal Kinetic Distraction (ISKD).

Methods: In this retrospective study, 12 consecutive ISKD procedures were performed for femoral lengthening and followed for an average of 76 months. After the ISKD group, 20 consecutive femoral lengthening procedures were performed as an LON technique and followed for an average of 27 months.
Results: There was no significant difference in achieving the lengthening goals between the two procedures. The healing index for the LON group averaged 1.4 months/cm, while the ISKD group was 3.2 months/cm (p=0.242). The distraction rates for the ISKD had a fast group (>1mm/day) with an average distraction rate of 1.7 mm/day and a slow group (<1mm/day) with a distraction rate of 0.84 mm/day. The LON group had an average distraction rate of 0.88 mm/day (p<0.001). The incidence of complications that required further unanticipated surgeries for the LON group was 1/20 (5%), while the ISKD group had complications in 6/12 femurs (50%, p=0.004).
Conclusions: We concluded that the LON technique is a more predictable and reliable method for femoral lengthening than the ISKD.

P027
THE THROMBOGENICITY OF CELL SALVAGE IN ELECTIVE HIP SURGERY?
PG Alexander*[1], I Ford[1], GP Ashcroft[1], HG Watson[2]
[1]School of Medicine and Dentistry, University of Aberdeen, UK;[2]Department of Haematology, Aberdeen Royal Infirmary, UK
The reinfusion of perioperative cell salvage is one method employed to reduce exposure to donor blood. Data on the safety of this process, however, are scant. Notably, the effect of intraoperative, washed cell salvage reinfusion on prothrombotic markers has not been demonstrated. The risk of postoperative venous thromboembolism following major orthopaedic operations is not insignificant. The study objective was to assess the effect of cell salvage reinfusion on coagulation and platelet activation.
Twenty-one patients undergoing elective primary hip operations were recruited. Nine patients received washed cell salvage intraoperatively, and were compared with 12 patients undergoing similar surgery that did not. Two patients in the cell salvage group also received postoperative, unwashed cell salvage. Blood samples were collected pre-operatively, immediately post-operatively, and one day post-operatively for assays of platelet activation markers, P-selectin expression and fibrinogen binding by flow cytometry in diluted whole blood; coagulation activation marker, thrombin-antithrombin complex (TAT); D-dimer by ELISA, thrombin generation by chromogenic assay, and full blood count. Samples of cell salvage material were also analysed for prothrombotic markers.
There were no significant differences between the groups preoperatively. Postoperatively haemoglobin levels did not differ significantly between the cell salvage group and controls. Postoperative TAT and D-dimer were significantly higher in the cell salvage group compared with controls (p<0.05). One day postoperatively, there were significantly higher platelet P-selectin expression (p=0.006) and platelet fibrinogen binding (p=0.004) in the cell salvage group compared with controls. The white cell count (WCC) was also significantly higher (p=0.04). In the intraoperative washed cell salvage material, and in postoperative cell salvage, the platelet count was low, but significant proportions of platelets were activated, and levels of D-dimer were elevated compared with venous blood. The postoperative salvage material also contained high levels of TAT.
The results from this pilot study show the induction of a prothrombotic state following reinfusion of intraoperative, washed cell salvage in recipients undergoing primary elective hip operations. An inflammatory response to reinfusion is also indicated by the raised WCC. Further investigation into the safety of cell salvage is indicated.

P028
RICKETS INDUCES SIGNIFICANT DETERIORATION IN NANOMECHANICAL QUALITY THROUGH INCOMPLETE EXTRAFIBRILLAR MINERALIZATION: EVIDENCE FROM IN-SITU SYNCHROTRON X-RAY SCATTERING AND BACKSCATTERED ELECTRON IMAGING
A Karunaratne*[1], C Esapa[2], A Boyde[3], J Hiller[4], N Terril[4], S Brown[5], R Cox[5], A Barbar[1], RV Thakker[5], HS Gupta[1]
[1]School of Engineering and Material Science, Queen Mary University of London, UK; [2]Bone and Mineral Disorders MRC Mammalian Genetics Unit, Harwell, Oxfordshire, UK; [3]Biophysics, Oral Growth and Development, Barts and The London, Queen Mary, University of London; [4]Beam Line I22- Non Crystaline Diffraction, Diamond Light Source Ltd. Diamond House, Didcot, Oxfordshire, UK; [5]Oxford Centre for Diabetes, Endocrinology & Metabolism Churchhill Hospital Headington Oxford, UK
Metabolic bone disorders like rickets cause significant reduction in bone material quality and mechanical properties, as well as rate of skeletal development. However, the ultrastructural mechanism by which altered mineralisation causes a reduction in mechanical properties has not been investigated. We measured the in-situ fibrillar level deformation as a function of both disease condition and age in a novel murine rickets model termed Elvis created using ethyl-nitrosurea (ENU) mutagenesis. The effective fibril elastic modulus, ultimate yield strength and maximum fibril yield strain were measured for femora from mice at different stages of development in both rickets and wild-type strains. Time-resolved in-situ synchrotron small angle X-ray scattering technique was used to measure changes in the fibril D-period during applied tensile loading. The mineral content in the femora were estimated independently using backscattered scanning electron imaging (qBSE) on the same samples. We found a significant reduction of fibril modulus as well as an enhancement of maximum fibril strain for the rickets condition. Both modulus and maximum fibril strain increased consistently with age within both the rickets and wild-type series. The mean mineral content was on average 21 % less for the rickety than for the wild-type condition, and is far more heterogeneous. We propose a simple nanostructural model to explain these results, where partial or incomplete mineralisation of the extrafibrillar mineral coating on mineralised collagen fibrils in rickets leads to the reduced modulus and increased ultimate fibrillar strain, which is found to be in reasonable agreement with experiment.

P029
ANGIOGENIC POTENTIAL OF HUMAN DENTAL PULP STEM CELLS FOR SKELETAL TISSUE ENGINEERING
N AL-Hazaimeh*[1], J Beattie[2], M Duggal[3] and XB Yang[1,4]
[1]Biomaterials and Tissue Engineering Group, Leeds Dental Institute, University of Leeds, Leeds LS2 9LU, UK; [2]Department of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds LS2 9LU, UK; [3]Department of Pediatric Dentistry, Leeds Dental Institute, University of Leeds, Leeds LS2 9LU, UK; [4]NIHR Leeds Musculoskeletal Biomedical Research Unit, UK.
Angiogenesis and the ability to provide appropriate vascular supply are crucial for skeletal tissue engineering. The aim of this study was to investigate the angiogenic potential of human dental pulp stromal cells (HDPSCs) and stro-1 positive populations as well as their role in tissue regeneration (the clinical reality).
HDPSC were isolated from the pulp tissues of human permanent teeth by collagenase digestion. STRO-1 positive cells were enriched using monoclonal anti- STRO-1 and anti- CD45 PE conjugated antibodies together with and fluorescence activated cell sorting (FACS). Cells isolated by FACS were grown to passage4 and cultured as monolayers or on 3D Matrigel scaffold in endothelial cell growth medium-2 (EGM-2) with/without 50ng/mL of vascular endothelial growth factor (VEGF). Cells cultured in alpha MEM supplemented with 10% FCS were used as controls. After 24, 48 and 72 hours angiogenic marker expression (CD31, CD34, vWF and VEGFR-2) was determined by qRT-PCR and immuno-histochemistry.
Using three different donors, 0.5-1.5% of total HDPSCs population was characterized as STRO-1+/CD45- cells At each time point cells cultured as monolayer in EGM-2 with VEGF showed up regulation of CD31 and VEGFR-2 expression compared to the control group while expression of CD34 and vWF remained unaffected. However on Matrigel, all four genes were up regulated to different extents. CD31 and VEGFR-2 were up regulated to a greater degree compared to CD34 and vWF. Changes in gene expression in both cell types were time dependent. Immuno-histochemical staining confirmed that the HDPSCs cultured in the test group showed positive staining for the four angiogenic markers (CD31, CD34 vWF and VEGFR-2) when grown in both monolayer and 3D Matrigel culture compared to control cultures. When cultured on Matrigel (but not Monolayer) for 7 days, HDPSC formed tube-like structures in the VEGF treated group.
This indicates the potential of use HDPSCs and their STRO-1 positive population for angiogenesis to enhance skeletal tissue repair and/or regeneration toward translational research for clinical benefit.

P030
GROWING THE SPINE STRAIGHT MECHANICAL TESTING OF A PROTOTYPE ANTI- SCOLIOSIS DEVICE
TS Drew*[1], JNA Gibson[2], J Burke[3]
[1]Department of Orthopaedic and Trauma Surgery, University of Dundee, UK; [2]Department of Orthopaedics, University of Edinburgh, UK; [3]Galway Clinic, Doughista, Galway, Eire
Growth rods are currently used in young children to hold a scoliosis until the spine has reached a mature length. Only partial deformity correction is achieved upon implantation, and secondary surgeries are required at 6-12 month intervals to lengthen the holding rod as the child grows. This process contains, rather than corrects, the deformity and spinal fusion is required at maturity. This treatment has a significant negative impact on the bio-psychosocial development of the child.
To design a device that would provide a single minimally invasive, non-fusion, surgical solution that permits controlled spinal movement and delivers three dimensional spinal correction.
Physical and CAD implant models were developed to predict curve and rotational correction during growth. This allowed use of static structural finite element analysis to identify magnitudes and areas of maximum stress to direct the design of prototype implants. These were mechanically tested for strength, fatigue and wear to meet current Industrial standards.
A dynamic hinged construct, was produced. This consisted of carbon nitride coated CoCrMo components assembled in a modular fashion. Five implants were tested under static load to simulate spinal flexion establishing a mean average yield point at a bending moment of 20.8 Nm (SD 2.5 Nm). Six samples were tested for fatigue endurance to 10 million cycles. Two implants were

loaded with a 10 Nm maximum bending moment without fracture. Two samples were loaded at 14 Nm with one surviving and one fracturing at 569,048 cycles. Samples loaded at 16 Nm and 17 Nm both fractured at 3,460,359 and 237,613 cycles respectively. Two implants were tested for wear, the first fractured after 290,000 cycles. A second modified implant was tested to ten million cycles and a mean wear rate of 2.03 mg per million cycles was determined during this period. Exposure of the CoCrMo implant substrate was first observed at two million cycles.
The device met all mechanical test criteria necessary for CE marking and allowed progression to implant testing in an ovine model.

P031
FAILURE OF CELLULAR PROCESSES IN BONE: IMPLICATIONS FOR REMODELLING
C Dooley*[1], D Cafferky[1], TC Lee[1,2], D Taylor[1]
[1]Trinity Centre for Bioengineering, Trinity College, Dublin, Ireland; [2]Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland
Bone sustains mechanical damage in the form of microcracks, as a result of everyday loading activities. If not repaired, these cracks grow to cause stress fractures in athletes and fragility fractures in people with osteoporosis.
Previously, we have proposed that microcracks in bone could be detected by the osteocyte network, via a mechanism in which cellular processes, residing in canaliculi, are cut where they cross the crack faces. The present work provides more support for this so-called 'Scissors' mechanism.
We examined large numbers of cracks in bone samples ex vivo and after mechanical testing. In smaller cracks (less than 100 microns long), subjected to in vivo load levels, cellular processes passed across faces and remained intact. With increasing crack length, larger numbers of broken processes occurred. We found that when higher stresses were applied, more processes ruptured, and that the mode of fracture was fatigue (due to repeated cycles) rather than monotonic rupture during a single load application.
We cultured osteocyte-like cells under conditions in which they formed networks, connected by processes. We simulated a microcrack by scratching with a thin wire. We observed a large increase in the number of molecules of the cytokine RANKL within one hour of damaging the network.
These results have quantified, for the first time, the number of cellular processes which rupture, or remain intact, where they cross microcracks. The data prove conclusively that the number of ruptures is directly related to the severity of crack length and applied stress, showing that it could be used as a mechanism to detect cracks which need to be repaired by bone's remodelling system. Our cell experiments are significant because they demonstrate a possible signalling pathway: RANKL is known to stimulate osteoclast differentiation, which is the first stage of bone remodelling.

P032
THE EFFECT OF CEMENT VISCOSITY ON MECHANICAL BEHAVIOUR IN A VERTEBROPLASTY MODEL
JRM Craig*[1], F Buchanan[2], R O'Hara[2], N Dunne[2]
[1]Trauma & Orthopaedic Department, Altnagelvin Hospital, Londonderry, UK; [2]School of Mechanical and Aerospace Engineering, Queen's University Belfast, UK
Vertebroplasty is a minimal invasive surgical procedure for treatment of vertebral compressive fractures, whereby cement is injected percutaneously into a vertebral body. Cement viscosity is believed to influence injectability, cement wash-out and leakage. Altering the liquid to powder ratio can affect the viscosity, level of cohesion and extent cement fill within the vertebral body and the ultimately strength and stiffness of the cement-vertebra composite. The association of these combined factors remains unclear. The aim of this study was to determine the relationship between cement viscosity and the potential augmentation of strength and stiffness in a model simulating in-vitro prophylactic vertebroplasty of osteoporotic vertebral bodies.
Samples of synthetic bone (Sawbone) representing osteoporotic bone were manually injected with 1mL of calcium phosphate cement using a 11G cannulated needle. Calcium phosphate cement was produced by mixing alpha-tricalcium phosphate, calcium carbonate and hydroxyapatite with an aqueous solution of 5 wt% disodium hydrogen phosphate. Three liquid to powder ratio (LPR) representing different viscosity levels were used; i.e. 0.5mL/g (low viscosity), 0.45mL/g (medium viscosity) and 0.35mL/g (high viscosity). Cement filled samples were then placed in an oven (37oC) for 20 min and then immersed in Ringer's solution (37oC) for 3 days. Samples of synthetic bone without cement injection were used as controls.
Potential for leakage and wash-out was determined using gravimetric analysis. Extent of cement fill was determined using computer tomography (CT).
Samples were tested under axial compression at a rate of 1 mm/min and the strength and stiffness determined. Statistical significance against controls was determined using a one-way analysis of variance ($p<0.05$).
Low viscosity cement showed more cement leakage ($p=0.512$) and increased cement wash-out after 3 days in Ringer's solution ($p=0.476$). Qualitative assessment of cement fill within the vertebral body using CT imaging supported the wash-out results. The strength ($p<0.05$-0.01) and stiffness ($p<0.01$) of samples significantly increased by cement injection in comparison to control, the extent of this increase was greater with increasing cement viscosity.
Linear correlation analysis showed a definite association between the mechanical properties and viscosity of injected cement and was dependent on the amount of cement retained within the synthetic bone post-setting.

P033
A NEW APPROACH TO MODELLING BONE REMODELLING: SMOOTHED PARTICLE HYDRODYNAMICS (SPH)
CDL Thomas*[1], JW Fernandez[2], R Das[2], JG Clement[1]
[1]The Melbourne Dental School, University of Melbourne, Australia; [2]Auckland Bioengineering Institute, University of Auckland, New Zealand
Introduction: There have been several approaches taken towards creating a mathematical model of bone remodelling. These have been limited to abstract formulations that describe overall bone loss over time without taking into account local variations in the strain field within the bone. A deeper understanding of age-related changes in the bony cortex is essential if an understanding of the mechanisms by which bones weaken and become vulnerable to fracture is to be gained. The aim of this study was to (i) examine a hypothesis that strain fields arise due to age related pore distributions generating low strain fields and bone resorption, and (ii) use a meshless Lagrangian particle-based computational modelling approach, Smoothed Particle Hydrodynamics (SPH), to represent bone remodelling effectively.
Materials and methods: A two-dimensional model was generated from a contact microradiograph of a section of human femoral cortex. The sample displayed the characteristics of bone from a healthy individual in mid-life. Using SPH software developed by the Auckland Bioengineering Institute authors the model was loaded with a compressive force to simulate static weight bearing. As a first experiment the amount of local strain modification in areas close to Haversian canals were recorded. Secondly, values of strain-dependent bone removal and apposition rates were taken from the literature and particles removed or added to the bone surfaces in the model depending upon the local strains. The model was run again and the changes in bone quantity and in the local strain modifications noted. This process was repeated through four cycles.
Results: The key findings from this study were firstly that bone remodelling due to reduction in strain at the osteon level generated pore merging and this increased with age. Secondly, SPH was shown to be effective at modelling the removal and deposition of bone and the intricate pore shapes that evolved over time.
Conclusion: Smoothed Particle Hydrodynamics provides a powerful new method for the mathematical modelling of bone remodelling. It offers the ability to add and remove particles of bone at locations determined by both global and local strains in models based on images (2D and 3D) of real bone.

P034
CIRCULATING OSTEOPROTEGERIN, SOLUBLE RANK LIGAND, IL-17A AND COMP LEVELS IN RELATION TO BONE MINERAL DENSITY IN PATIENTS WITH SYSTEMIC SCLEROSIS
K Simic Pasalic*[1], N Damjanov[1], N Pejnovic[1], I Soldatovic[2]
[1]Institute of rheumatology, Belgrade, Serbia; [2]Institute of Medicine Statistics, School of Medicine, University of Belgrade, Serbia
The imbalance in RANK/RANKL/OPG system affects bone remodeling. Data concerning bone mineral density (BMD) in SSc pts are discrepant.
To evaluate the relationship between serum osteoprotegerin (OPG), soluble RANK ligand, IL-17A and cartilage oligomeric matrix protein (COMP) levels and BMD in patients with SSc.
Forty pts with SSc, 22 limited, 18 diffuse subtype (38 females), age 55,5yrs (30-79), disease duration 6 yrs (1-28), with early SSc (<5yrs-20pts), late SSc (>5 yrs-20pts) and 40 healthy controls matched for sex,age , menopause
duration and BMI, were analysed for BMD, T-scores of lumbar spine and total hip by dual energy X-ray absorptiometry
using Lunar Prodigy device. Serum levels of various soluble mediators were determined by ELISA.
Fourteen SSc patients presented osteopenia and 10 densitometric osteoporosis and those frequencies were not significantly different from healthy controls ($p=0.378$). There were no differences in age, gender, duration of menopause, body mass index among patients with early or late SSc and healthy controls. BMD and T-scores of lumbar spine and total hip were not significantly different between SSc patients and controls. However, lumbar spine BMD(0.96±0.16 vs. 1.11±0.19, $p=0.056$) and T-score (-1.89±1.50 vs. -0.54±1.61, $p=0.039$) were significantly lower in patients with late compared to early SSc. Serum OPG levels were similar in SSc patients and healthy controls, while sRANKL(0.217 pmol/L±0.346 vs. 0.055 pmol/L ±0.071, $p=0.033$) and sRANKL/OPG ratio (0.007±0.010 vs. 0.020±0.029, $p=0.080$)were lower in patients with early SSc compared to controls. The serum level of IL-17A was lower in patients with early(1.6pg/ml±5.6) and late SSc (9.7pg/ml±20.7) than in controls (57.9 pg/ml±45.9, $p=0.000$ for both). Serum COMP levels were elevated in patients with early (2467.9 ng/ml± 647.3)) and late SSc (2305.3 ng/ml±468.8) compared to controls(1927.4 ng/ml±417.7, $p=0.001$ and $p=0.005$, respectively). In healthy controls, serum OPG and COMP levels negatively correlated with lumbar spine and total hip BMD and T-scores, whereas in SSc patients the negative

correlation was noticed between serum OPG levels and lumbar spine BMD and T-scores.
The present study show that SSc patients have a similar frequency of OP, BMD and T-score values as
healthy controls. Decreased
serum sRANKL, IL-17A, lower sRANKL/OPG ratio and increased COMP levels in patients with early SSc compared to
healthy controls might be involved in protection against bone loss in SSc.

P035
ASSESSMENT OF FEMORAL HEADS FROM DIFFERENT SPECIES FOR USE IN AN IN-VITRO SIMULATION
SD Taylor*[1], E Tsiridis[2], E Ingham[1], Z Jin[1], J Fisher[1], S Williams[1]
[1]University of Leeds, Leeds, UK; [2]Leeds General Infirmary, Leeds, UK
Tribology and wear of articular cartilage is associated with the mechanical properties, which are governed by the extracellular matrix (ECM). The ECM adapts to resist the loads and motions applied to the tissue. Most investigations take cartilage samples from quadrupeds, where the loading and motions are different to human. However, very few studies have investigated the differences between human and animal femoral head geometry and the mechanical properties of cartilage.
This study assessed the differences between human, porcine, ovine and bovine cartilage from the femoral head; in terms of anatomical geometry, thickness, equilibrium elastic modulus and permeability.
Diameter of porcine (3-6 months old), bovine (18-24 months old), ovine (4 years old) and human femoral heads were measured (n=6). Plugs taken out of the superior region of each femoral head and creep indentation was performed. The human femoral heads were obtained from surgery due to femoral neck fracture. Cartilage thickness was measured by monitoring the resistive force change as a needle traversed the cartilage and bone at a constant feed rate using a mechanical testing machine. The percentage deformation over time was determined by dividing deformation by thickness. A biphasic finite element model was used to obtain the intrinsic material properties of each plug. Data is presented as the mean ± 95% confidence limits. One-way ANOVA was used to test for significant differences ($p < or = 0.05$).
Significant differences in average femoral head diameter were observed between all animals, where bovine showed the largest femoral head. Human cartilage was found to be significantly thicker than cartilage from all quadrupedal hips. Human cartilage had a significantly larger equilibrium elastic modulus compared to porcine and bovine cartilage. Porcine articular cartilage was measured to be the most permeable which was significantly larger than all the other species. No significant difference in permeability was observed between human and the other two animals: bovine and ovine (Table 1).
The current study has shown that articular cartilage mechanical properties, thickness and geometry of the femoral heads differ significantly between different species. Therefore, it is necessary to consider these variations when choosing animal tissue to represent human.

P036
PREDICTING THREE-DIMENSIONAL FEMORAL OFFSET FROM AP PELVIS RADIOGRAPHS IN PRIMARY HIP OSTEOARTHRITIS
C Merle*[1,2], W Waldstein[1,2], E Pegg[1], MR Streit[2], T Gotterbarm[2], PR Aldinger[3], DW Murray[1], HS Gill[1]
[1]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, UK; [2]Department of Orthopaedic Surgery and Traumatology, University of Heidelberg, Germany; [3]Department of Orthopaedic Surgery, Paulinenhilfe, Diakonieklinikum Stuttgart, Germany
In pre-operative planning for total hip arthroplasty (THA), femoral offset (FO) is frequently underestimated on AP pelvis radiographs as a result of inaccurate patient positioning, imprecise magnification, and radiographic beam divergence. The aim of the present study was to evaluate the reliability and accuracy of predicting three-dimensional (3-D) FO as measured on computed tomography (CT) from measurements performed on standardised AP pelvis radiographs.
In a retrospective cohort study, pre-operative AP pelvis radiographs and corresponding CT scans of a consecutive series of 345 patients (345 hips, 146 males, 199 females, mean age 60 (range: 40-79) years, mean body-mass-index 27 (range: 29-57) kg/m2) with primary end-stage hip osteoarthritis were reviewed. Patients were positioned according to a standardised protocol and all images were calibrated. Using validated custom programmes, FO was measured on corresponding AP pelvis radiographs and CT scans. Inter- and intra-observer reliability of the measurement methods were evaluated using intra-class correlation coefficients (ICC). To predict 3-D FO from AP pelvis measurements, the entire cohort was randomly split in two groups and gender specific linear regression equations were derived from a subgroup of 250 patients (group A). The accuracy of the derived prediction equations was subsequently assessed in a second subgroup of 100 patients (group B).
In the entire cohort, mean FO was 39.2mm (95%CI: 38.5-40.0mm) on AP pelvis radiographs and 44.6mm (95%CI: 44.0-45.2mm) on CT scans. FO was underestimated by 14% on AP pelvis radiographs compared to CT (5.4mm, 95%CI: 4.8-6.0mm, $p<0.001$) and both parameters demonstrated a linear correlation ($r=0.642$, $p<0.001$). In group B, we observed no significant difference between gender specific predicted FO (males: 48.0mm, 95%CI: 47.1-48.8mm; females: 42.0mm, 95%CI: 41.1-42.8mm) and FO as measured on CT (males: 47.7mm, 95%CI: 46.1-49.4mm, $p=0.689$; females: 41.6mm, 95%CI: 40.3-43.0mm, $p=0.607$).
The results of the present study suggest that femoral offset can be accurately and reliably predicted from AP pelvis radiographs in patients with primary end-stage hip osteoarthritis. Our findings support the surgeon in pre-operative templating and may improve offset and limb length restoration in THA without the routine performance of CT.

P037
HTRA1 AND TYPE VI COLLAGEN ARE UPREGULATED BY SINGLE IMPACT LOAD IN A NOVEL MODEL OF EARLY OA IN HUMAN CARTILAGE
FMD Henson*, P Hernandez, J Wardale, N Rushton
Cambridge, UK
Introduction: HTRA1, a serine protease, has been shown to be upregulated in osteoarthritis (OA). It has been proposed that HTRA1 protein is upregulated as an initial event in OA which leads to breakdown of type VI collagen, an important pericellular matrix component. In order to investigate the relationship of HTRA1 and type VI collagen in the early events of human OA this work aims to i) characterise a single impact load (SIL) model of cartilage damage in human tissue and ii) describe the immunolocalisation of HTRA1 and type VI collagen in this model.
Materials and Methods: 7mm full thickness cartilage discs were obtained from normal hips obtained from patients with femoral neck fractures (n=4). Cartilage was SIL with 250g from various heights, cultured for up to 10 days and processed for routine histology. Immunohistochemistry was performed using anti-HTRA1 and anti-type VI antibodies.
Results: 2.5cm was chosen as the height from which SIL of 250g should be applied to mimic tissue damage seen in OA as it causes characteristic tissue damage (surface lamination, fissure formation, loss of toluidine blue and apoptosis at the surface of the cartilage). HTRA1 immunoreactivity was not present in normal cartilage at t = 0 days and was seen in the surface zone of control cartilage at 4d of culture onwards. In SIL cartilage HTRA1 was detected in the surface zone at day 1 and throughout the cartilage by day 10. Type VI collagen was detected in the surface and mid zone in control cartilage and strongly throughout the cartilage in SIL samples. HTRA1 and type VI collagen were shown to co-localise in a number of chondrocytes.
Discussion: This study describes a SIL model of early OA in human cartilage. In this model it is demonstrated that HTRA1 and type VI collagen immunoreactivity is increased after SIL and culture and that the HTRA1 and type VI collagen can be co-localised. These results challenge previous findings by other workers in murine models and suggest an alternative hypothesis for the role of HTRA1 in OA.

P038
A CADAVERIC MODEL FOR SUPRASCAPULAR NERVE INJURY DURING GLENOID COMPONENT SCREW INSERTION IN REVERSE GEOMETRY SHOULDER ARTHROPLASTY
DC Molony, AJ Cassar Gheiti*, J Kennedy, C Green, A Schephens, HJ Mullet
Dublin, Eire
Reverse Geometry shoulder replacement requires fixation of a base plate (called a metaglene) to the glenoid to which a convex glenosphere is attached. Most systems use screws to achieve this fixation. The suprascapular nerve passes close to the glenoid and is known to be at risk of injury when devices and sutures are inserted into the glenoid. We investigate the risk posed to the suprascapular nerve by placement of metaglene fixation screws.
Ten cadaveric shoulder specimens were used. A metaglene was inserted and fixed using 4 screws. The suprascapular nerve was dissected and its branches identified. The screw tips and their proximity to the nerve and branches were identified and recorded.
The superior and posterior screws posed most risk to the suprascapular nerve. The nerve was engaged by the posterior screw on 4 occasions and was within 5 mm of the nerve or a branch of it in 5 others. The superior screw was extra osseous on 4 occasions, making contact with the nerve on 3 of those 4 specimens and being within 2 mm of it on the 4th.
Metaglene fixation using screws poses a significant risk to the suprascapular nerve. Caution should be used when inserting the posterior and superior screws in particular. Short locking screws may allow adequate fixation while minimizing the risk of neurological injury.

P039
STUDY OF FAILED OXFORD UNICOMPARTMENTAL KNEE REPLACEMENTS AT THE ROYAL CORNWALL HOSPITAL
S Sarkar*[1], M Divekar[1]
[1]Royal Cornwall Hospital, Truro, UK
Objective - we set up a retrospective study to identify the various reasons for failure of oxford medial unicompartmental knee replacements and to assess their outcome following revision.
Materials and Method- Over 5 years (2006- 2010) we identified 26 failed unicompartmental knee replacements, which were revised at the Royal Cornwall hospital. We retrospectively analysed the data to include pre-operative

and post-operative Oxford score, range of movement, patient satisfaction and the type of implant used.
Results- There were 9 males and 17 females in our series with an average age of 65 years (49 to 80). The average follow up was 2.6 years (1 - 4.6 years). The pre-revision Oxford score was 21.3 (12 to 35), which improved to 41.7 (18 to 47) following surgery. Almost all patients benefited with increase in the range of movement. The implants were revised at an average duration of 4 years and 8 months (1 to 17 yrs) following the index operation. The commonest cause of failure was progression of arthritis in the lateral compartment 50 % (13/26), revision for unexplained pain 23 % (6/26) and aseptic loosening 23 % (6/26). There was one case of sepsis 4 % (1/26). We did not come across dislocation of the bearing. The implants were revised using primary or complex primary knee systems. The infected knee was revised using a two-stage technique.
Conclusion- Unicompartmental knee replacement is a successful procedure for treating isolated medial compartment arthritis. Commonest indication for revision in our study was progression of arthritis in the lateral compartment. Revision is relatively easy and results of revision are good with high patient satisfaction. Our results are comparable to published data from larger centres.

P040
Abstract withdrawn

P041
THE OVINE CALCANEUS: A USEFUL MODEL FOR FRAGILITY FRACTURES OF THE HIP?
N Loveridge[1], J Power[1], J Reeve[1], M Warren[2], M Doube[3], A Goodship[2]
[1]Orthopaedic Research Unit and Dept of Medicine, University of Cambridge; [2]Royal Veterinary College, London; [3]Imperial College, London, UK
Cases of fragility hip fracture, show excessive loss of cancellous connectivity, cortical thinning and increased porosity. While alterations in mechanical loading are considered to play a key role, currently there is no satisfactory animal model, which mimics the essential biomechanics of the human femoral neck and allows the assessment of both intra-cortical and cancellous bone turnover. The ovine calcaneus is a short bone cantilever loaded in bending with a defined cancellous architecture and cortices that undergo intra-cortical adaptation to habitual tensile and compressive loads. We hypothesised that this bone would respond to decreases in loading with changes in both cancellous and cortical bone so providing an appropriate model to study fragility fractures of the hip. Using external fixators to protect the left calcaneus of ewes (6/group) from normal levels of load related strain we analysed the consequent changes in cortical and cancellous bone after 4, 8 & 16 weeks using the contra-lateral bone as a control.
Fixation reduced maximum local loads by 50% (free: 95.9 microstrain±24.4 (SD); fixed: 48.0±9.9 (SD)) but the marked individual patterning between bones from the same animal was unaffected. Bone volume and BV/TV reduced over time in the left (load protected) but not the right (control) calcaneus (BV: Left -34.72mm3/wk p=0.0002; Right: -4.83mm3/wk, p=0.555 p<0.0001; BV/TV: Left -0.0069/wk; Right: - 4.1522e-5/day, p=0.80).
Trabecular thickness, and number, but not spacing, was affected (Tb.Th: Left -0.133mm/wk, p<0.0001; Right: +0.0084mm/wk, p=0.657; 1/TbN: Left: +0.0133/wk, p<0.0001; Right: +52.87e-5/wk, p=0.75). Both SMI (Left +0.437/wk p<0.0001; Right: -0.11/wk, p=0.339) and aniostropy (Left -0.442/wk p<0.0001; Right: +0.0149mm/wk, p=0.657) were altered by under-loading.
The %cortical area (diff; - 10.6%, p=0.012) and cortical thickness (diff -0.13mm p=0.018) were reduced in the under-loaded calcaneus at 16 weeks. Mean canal area was increased (diff +62.5±15um2; p=0.02) but canals/bone area was unchanged.
In conclusion, under-loading the ovine calcaneus resulted in changes in cancellous and cortical bone, which mimicked most of those seen in fragility fractures of the human femoral neck. This suggests that this model is suitable to further explore hypotheses related to the causes of intracapsular hip fracture and the development of new prevention strategies.

P042
ELECTROCHEMICAL TREATMENT OF TITIANIUM INFLUENCES OSTEOBLAST ACTIVITY
SE Johnson-Lynn*, A McCaskie, MA Birch
Musculoskeletal Research Group, Institute of Cellular Medicine, University of Newcastle, UK
Uncemented implants are an important part of the arthroplasty armamentarium. Risk of aseptic loosening and failure of these components is related to initial osseointegration - the formation of a seamless bone-implant interface without interposition of fibrous tissue.
Modification of the surface properties of titanium alloy, to enhance suitability for early osseointegration.
Samples of Ti6Al4V were prepared with different surface finishes: machined; polished with grit papers to a mirror finish or treated in an electrochemical cell with sulphuric acid/methanol electrolyte using 3, 5 or 9V for 60, 120 or 180 seconds . Electrochemical modification produced average roughness (Ra) values, which differed significantly between the 3 different voltages applied (p<0.05) with those treated at 3V being the roughest and those at 9V the smoothest.
Rat osteoblasts and human mesenchymal cells were cultured on the samples for 24 hours and 48 hours respectively. Immunofluorescence was performed to localise vinculin, elucidating cell morphology and identifying focal adhesion complexes.
Surface modification created quantifiable differences in morphology of rat osteoblasts. Rat cells on Ti6Al4V treated with 3V and 5V were significantly more polarised than those on 9V, glass and polished control surfaces (p=<0.05). This behaviour can, in part, be explained by differences in size and distribution of focal adhesions, which act as anchor points for cell adhesion. There is a trend for lower density of focal adhesions on the surfaces treated with 3V and 5V compared to those treated with 9V and the control surfaces, with some comparisons reaching statistical significance (3V180s, 5V60s and 5V120s vs 9V120s p=<0.05).
These differences were also seen with human cells. Those on the 3V and 5V surfaces were significantly more polarised (p<0.05) than those on the 9V and control surfaces. Focal adhesion area was also significantly lower on 3V and 5V surfaces compared with glass and 9V surfaces.
Preliminary results from long term culture of rat osteoblasts show greater areas of bone nodule formation on surfaces modified with higher voltages for longer time periods.
Electrochemical modification of titanium alloy alters morphology and adhesion-related behaviour of rat and human osteoblasts, which influences differentiation and osteogenesis.

P043
VITAMIN D3-STIMULATED BONE FORMATION IN AN ORGANOTYPIC EX VIVO CULTURE SYSTEM OF CHICK FEMORA
EL Smith*, JM Kanczler, CA Roberts, ROC Oreffo
Bone and Joint Research Group, University of Southampton, Southampton, UK
Enhancement and application of our understanding of human skeletal developmental biology is critical to developing new tissue engineering approaches to skeletal repair. We propose that the use of the developing fetal femur to understand the differentiation of skeletal stem cells, skeletogenesis, and the effects of key differentiation agents, will aid our understanding of the developing bone and niche, providing a unique regenerative medicine paradigm. We have used a novel three dimensional organotypic culture system of embryonic chick femora, to investigate the effects of 1alpha,25-dihydroxyvitamin D3 on bone and cartilage development.
Embryonic chick femurs from E11 were harvested and placed in organotypic cultures for 10 days in basal media alone, or basal media supplemented with 25uM vitamin D3. Organotypic cultures were analyzed by micro-computed tomography (microCT), and further assessed histologically for proteoglycan (alcian blue), collagen production (Sirius red), and mineralization (von Kossa).
Stimulation of organotypic cultures with vitamin D3 increased the chick femur length (mm) by 17.5%, from E11(basal)=10.3±0.4 to E11(vitamin D3)=12.1±0.3. MicroCT analysis (10um resolution) demonstrated that, compared to basal cultures, stimulation with vitamin D3 increased Bone Volume/Total Volume (BV/TV) by 55.5%, increased Trabecular Thickness (TbTh (mm3)) by 33.3%, increased Trabecular Number (TbNo (mm)) by 18.6%, and decreased Trabecular Spacing (TbSp (mm)) by 16%. Histological analysis demonstrated an increase in bone formation in response to vitamin D3 stimulation, as evidenced by a 49% increase in collagen production, and increased bone mineralization as evidenced by a 20% increase in positive von Kossa calcium staining.
This study demonstrates the successful use of organotypic chick femur cultures as a model system for bone development, evidenced by the effect of exogenous vitamin D3 to modulate bone formation. This organotypic model provides a tool for the temporal analysis of the key stages of bone and cartilage development, providing a paradigm for translation of bone development to improved scaffolds and skeletal stem cell treatments for regenerative medicine.

P044
ENDOGENOUS EXTRACELLULAR NUCLEOTIDES ARE IMPORTANT AUTOCRINE/PARACRINE REGULATORS OF BONE MINERALISATION
IR Orriss*, ML Key, TR Arnett
Department of Cell and Developmental Biology, University College London, London, UK
Extracellular nucleotides, signalling through P2 receptors, play an important role in bone biology, modulating both osteoblast and osteoclast function. Previous work has shown that exogenous ATP/UTP (>1micromolar) prevents bone formation in vitro by blocking mineralisation of the collagenous matrix. We have also demonstrated that osteoblasts constitutively release ATP in the range 0.5-10nmol/ml under normal conditions; this release is enhanced by a number of external stimuli including fluid shear stress and hypoxia. Primary osteoblast cultures were obtained from neonatal rat calvariae by trypsin/collagenase digestion and cultured for up to 14 days with 0.5-2.5U/ml apyrase (a broad spectrum ecto-nucleotidase which sequentially hydrolyses nucleotide tri- and di-phosphates to their corresponding nucleotide monophosphate and phosphate (Pi)). This study used biochemical assays and qPCR microarrays to investigate the role of endogenously produced nucleotides in osteoblast differentiation and function; mRNA expression and enzyme activity were studied at day 7 (differentiating osteoblasts) and 14 (bone-forming

osteoblasts). Addition of 0.5U/ml apyrase to culture medium rapidly (<1 minute) degraded the ATP present; within 2 minutes ATP levels were negligible. Long-term apyrase exposure resulted in extracellular ATP levels which were by more than 99% lower than normal. Indicating a proliferative role for ATP, apyrase caused small decreases (~25%) in osteoblast number for the first 72 hours of culture. Continuous apyrase treatment increased total bone formation by osteoblasts by up to 4.5-fold. Surprisingly, alkaline phosphatase (ALP) activity was decreased by up to 60% at both day 7 and 14. Conversely, the activity of the pyrophosphate (PPi) producing ecto-nucleotidases, E-NPPs (ecto-nucleotide pyrophosphatase/phosphodiesterase), was increased up to 2.7-fold. This resulted in a 2-3micromolar increase in increase medium PPi levels. In contrast, a single application of apyrase (0.5-2.5U/ml) decreased PPi levels by 3-4micromolar. Pi levels were consistently increased 10-15micromolar. No effects on collagen production and deposition were observed in these cultures. Analysis of mRNA showed that apyrase treatment did not affect the expression of ALP, E-NPP1, COL1 or osteocalcin, however, expression of SOST, MMP10 and TNF-alpha was down-regulated. These data indicate that, under normal conditions, endogenous nucleotides released by osteoblasts can act locally, probably via multiple mechanisms, to regulate bone formation.

P045

ADIPOSE-TISSUE DERIVED STEM CELLS IN MUSCULOSKELETAL REPAIR AND REGENERATION

NM Hopper*, J Wardale, N Rushton

Orthopaedic Research Unit, University of Cambridge, Box 180, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, UK

Mesenchymal stem cells (MSC) are an attractive cell population for regeneration of mesenchymal tissue such as bone and cartilage. Various studies have demonstrated the repair capacity of MSCs and even their usefulness in treating critical size defects. Much of the work conducted on adult stem cells has focused on MSCs found within the bone marrow stroma. Adipose tissue, like bone marrow, is derived from the embryonic mesenchyme and contains a stroma that is easily isolated. The aim of the present study is to evaluate the differentiation capability of adipose-tissue derived stem cells (ASC) extracted from the infrapatellar fat pad.

Human infrapatellar fat pad tissue was obtained from patients undergoing total joint replacement for osteoarthritis with full ethical consent. A multipotent progenitor cell population was derived after collagenase digestion from the adipose tissue. The ASCs were induced to differentiate towards adipogenic, chondrogenic, and osteogenic lineages for 21 days both in normoxic and hypoxic cell culture conditions. The differentiation and multilineage potential was assessed according to cell morphology and in vitro detection of tissue-specific differentiation molecules.

After 3 weeks in culture the staining for oil-red-o, alcian bue, and alizarin-red confirmed the differentiation capability of ASC's to adipogenic, chondrogenic, and osteogenic lineages, respectively. The hypoxic cell culture condition was found to support the ASCs' chondrogenic differentiation capability and subsequently enhanced the proteoglycan release from the cells. Fluorescence-activated cell sorting (FACS) confirmed the presence of stromal precursor cell marker STRO-1 in the ASC population.

Subcutaneous adipose tissue is particularly attractive reservoir for progenitor cells because it is easily accessible, rather abundant, and self-replenishing. The results of this study demonstrate that ASCs can be derived from infrapatellar fat pad and that they have potential for musculoskeletal tissue repair and regeneration. Further studies are underway to evaluate how to adopt a biomaterial to deliver these cells into the defect area to facilitate the healing response.

P046

SUBSTITUTE LUBRICANTS FOR WEAR TESTING ORTHOPEDIC BIOMATERIALS

SC Scholes*, TJ Joyce

Newcastle University, Newcastle, UK

Although bovine serum is the lubricant recommended by several international standards for the wear testing of orthopedic biomaterials there are issues over its use. The inherent batch variation in protein content means that two bovine serum lubricants can give different wear rates. Due to degradation, the lubricant needs to be changed regularly, so that any third body wear particles are removed, thus potentially influencing wear regimes. There are also cost and safety issues with the use of bovine serum. For these reasons, alternative lubricants were investigated.

A 50-station wear test rig was used, which applied multi-directional motion to each ultra-high molecular weight polyethylene (UHMWPE) test pin. Each pin articulated against a cobalt chrome plate polished to better than 0.05 microns Ra. The following lubricants were used: 50% dilute bovine serum; soy protein; olive oil; wheatgerm oil; soya oil; albumin and globulin (AG) mix; albumin, globulin and chondroitin sulphate (AGC) mix; whole milk; Channel Island milk; 11 mg/ml protein egg white; 20 mg/ml egg white; and 40 mg/ml egg white. A minimum of 6 UHMWPE pins per lubricant were wear tested and the tests ran to 2.5 million cycles. Gravimetric measurements were taken throughout the test to determine the volume of wear and at the end of the test the samples were examined using a SEM.

The lubricants giving the closest results to bovine serum were 20 and 40 mg/ml egg white, with mean UHMWPE total wear volumes of 17.4 mm3 and 17.8 mm3 compared to bovine serum which gave 20.7 mm3. Surface topographies showed similar features too. The 11 mg/ml egg white lubricant and the AG and AGC lubricants were next closest in terms of wear. An UV absorbance assay found that all the protein based lubricants suffered from a high degradation rate, and the rate increased with increasing protein content.

Egg white may offer a less expensive alternative to dilute bovine serum as a test lubricant although it is likely that it too would need to be changed as regularly as bovine serum.

P047

DOES THE DIAMETER OF THE INTRAMEDULLARY NAIL REALLY MATTER?

A J Howard*[1], L Neilson[2], G McLauchlan[3], J Richards[4], S Evans[5]

[1]Royal Liverpool Hospital, Liverpool, UK; [2]Manchester University, Manchester, UK; [3]Royal Preston Hospital, Preston, UK; [4]University of Central Lancashire, Preston, UK; [5]Cardiff University, Cardiff, UK

The fixation of comminuted femoral fractures with intramedullary nails is commonplace but there remains little work on the mechanical ability of the different diameters of nail available to resist bending. What previous work there is has produced conflicting conclusions. The bending stiffness against the intramedullary nail diameter and the extent of the comminuted fracture is clinically important due to the impact on fracture healing and implant failure.

Intramedullary nails of differing diameters (10 mm, 11 mm and 13 mm) were loaded axially in fourth generation composite femurs with increasing mid shaft bone defects, namely 3cm, 5cm, 8cm and 10cm bones. The loading versus the displacement was recorded for each nail.

A one-way ANOVA analysis demonstrated a significant difference between intramedullary nail diameters and the bending stiffness, with p values of less than 0.012; 3cm mean 12.26 (CI 9.06-15.46) mm, p=0.012; 5 cm mean 10.63 (CI 8.35-12.92) mm, p=<0.001; 8 cm mean 11.04 (CI 8.35-13.74) mm, p=<0.001; 10 cm mean 11.68 (CI 7.86-15.50) mm, p=<0.001. For the 11 mm diameter intramedullary nail, failure occurred at around two times the body weight of an average individual or 1400 to 1800 N. A repeated measure ANOVA analysis of the effect of the increasing bone defect showed a mixed picture, with a significant difference between the 5 cm and 8 cm gap and only a trend towards significance between 5 cm and 10 cm.

Caution should be advised when considering using a cannulated femoral intramedullary nail in a patient with a fracture gap of greater than 5 cm. Further, the mechanical effect of comminuted fractures treated with nails suggests reduced stiffness with increasing length of fracture gap although the picture is complex and explains the divergence of research conclusions.

P048

USE OF THIAZOLIDINEDIONES AND RISK OF OSTEOPOROTIC FRACTURE: THE DANISH NATIONAL DATABASES

A Lalmohamed*[1], MT Bazelier[1], A Gallagher[1,2], TP van Staa[1,3], C Cooper[3,4], HGM Leufkens[1], P Vestergaard[5], F de Vries[1,3]

[1]Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, NL; [2]General Practice Research Database, Medicines and Healthcare products Regulatory Agency, UK; [3]MRC Lifecourse Epidemiology Unit, University of Southampton, UK; [4]Institute of Musculoskeletal Sciences, University of Oxford, UK; [5]The Osteoporosis Clinic, Aarhus University Hospital, DK

Background: Clinical and observational studies have suggested that use of thiazolidinediones (TZDs) is associated with an increased fracture risk, in particular in women. Although Type 2 diabetes mellitus (T2DM) is a risk factor for osteoporotic fracture, it is unclear to which extent the association between TZD use and fracture risk is confounded by the severity of T2DM. TZDs are indicated in T2DM to delay the start of insulin treatment. If the disease severity would play a more prominent role than the use of TZDs, fracture risk in insulin users would be higher than in TZD users.

Objective: To evaluate whether the risk of osteoporotic fracture in TZD users is confounded by severity of the underlying type 2 diabetes mellitus.

Methods: We conducted a population-based cohort study using data from the Danish National Databases (1996-2007), which links pharmacy dispensing data to the national registry of hospitalisations and mortality. Oral antidiabetic users (n=180,049) were matched 1:4 by year of birth and sex to non-users. Cox proportional hazards models were used to estimate hazard ratios (HRs) of osteoporotic fracture in TZD users. Time-dependent adjustments were made for age, sex, comorbidity, and drug use. We created a proxy indicator for the severity of disease. The first stage of disease was defined as current use of either a biguanide or a sulfonyluerum, the second stage as current use of a biguanide and a sulfonyluerum at the same time, the third stage was assigned to patients using TZDs and the fourth stage to patients using insulin.

Results: The risk of osteoporotic fracture was increased 1.3-fold (HR 1.27, 95% CI 1.06-1.52) in patients who currently used TZDs (stage 3) and for patients using insulin (stage 4) the risk was increased 1.3-fold as well (HR 1.25, 95% CI 1.20-1.31), as compared with healthy controls. In the first and second stage risks were lower: HR 1.15 (1.13-1.18) for stage 1 and HR 1.00 (0.96-1.04) for stage 2.

Conclusion: Users of TZDs were at an increased risk of osteoporotic fracture. The association is probably partially confounded by the severity of type 2 diabetes mellitus.

P049
Abstract withdrawn

P050
MYASTHENIA GRAVIS AND RISK OF FRACTURE: THE GENERAL PRACTICE RESEARCH DATABASE
A Lalmohamed*[1], MT Bazelier[1], S Pouwels[1], MK Javaid[2], C Cooper[2,3], D Hilton-Jones[4], J Verschuuren[5], TP van Staa[1,6], F de Vries[1,3]
[1]Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, NL; [2]Institute of Musculoskeletal Sciences, University of Oxford, UK; [3]MRC Lifecourse Epidemiology Unit, University of Southampton, UK; [4]Department of Clinical Neurology, University of Oxford, UK; [5]Department of Neurology, Leiden University Medical Centre, NL; [6]General Practice Research Database, Medinices and Healthcare products Regulatory Agency, UK
Background: Myasthenia Gravis (MG) is a neuromuscular disease with symptoms of muscle weakness and fatiguability. MG has been associated with falling and glucocorticoid induced osteoporosis. Aim of this study was to evaluate the risk of fracture after onset of MG.
Methods: We conducted a retrospective cohort study (1987-2009, n=7,458) using the UK General Practice Research Database. Each MG patient was matched by age, sex, calendar time, and practice to up to 6 patients without a history of MG.
Results: No increased fracture risk was observed in patients with incident MG (adjusted hazard ratio "AHR" 1.11; 95% confidence interval "CI", 0.84 - 1.47). Use of oral glucocorticoids up to a cumulative dose exceeding 5 grams prednisolone equivalents did not further alter fracture risk (AHR 1.15 [95% CI, 0.40 - 3.30]). Fracture risk was doubled for recent use of antidepressants (AHR 1.81 [95% CI, 1.07 - 3.06]), anxiolytics/sedatives (AHR 1.97 [95% CI, 1.14 - 3.41]) and five-fold increased among recent users of anticonvulsants (AHR 4.98 [95% CI, 2.68 - 9.26]).
Conclusion: MG itself was not associated with increased fracture risk, except for patients using antidepressants, anxiolytics/sedatives or anticonvulstants. The use of (very high dosages of) oral glucocorticoids among MG patients did not alter fracture risk, for which the underlying mechanism remains unclear.

P051
INCREASED DIRECTIONALITY OF CORTICAL BONE STIFFNESS WITH OSTEOPOROSIS
P Pankaj*[1], FE Donaldson[1], DML Cooper[2], CDL Thomas[3], JG Clement[3], AHRW Simpson[1]
[1]Edinburgh Orthopaedic Engineering Centre, The University of Edinburgh; [2]College of Medicine, University of Saskatchewan; [3]School of Dental Science, The University of Melbourne
The effect of increased porosity (due to age or disease) on the directionality of cortical bone stiffness has not been discussed before. We evaluated the orthotropic elastic constants of cortical bone from the female anterior femoral midshaft using micro-finite-element analyses of bone samples from 27 female donors (age 53.4 +- 23.6). Analyses were also conducted to quantify the elastic constants at the periosteum and endosteum.
The ratio of canal volume to tissue volume (Ca.V/TV), analogous to porosity, was found to be the most significant predictor of the elastic constants. As would be expected stiffness decreased with increase in Ca.V/TV. Increased Ca.V/TV was found to result in greater reduction of elastic constants at the endosteal aspect than at the periosteal aspect. More interestingly, elastic anisotropy was seen to increase with increased Ca.V/TV. The longitudinal Young's modulus was found to decrease less rapidly with Ca.V/TV in comparison to the Young's moduli in the transverse directions. This increased directionality of stiffness is likely to have a direct bearing on the corresponding strengths, which can result increased vulnerability in the less frequently loaded (here transverse) directions.

P052
THE ROLE OF THE CALCAR FEMORALE IN HIP FRACTURE TYPE- A PILOT STUDY USING RAPID CT
PM Mayhew*, M Vindlacheruvu, KE Poole
*NIHR Biomedical Research Centre, University of Cambridge, Cambridge, UK
The calcar femorale or 'true neck' of the femur has a role in transmitting load from the cantilevered neck to the femoral shaft (Zhang 2009). It can appear as a distinct condensation in clinical CT images because its structure is very similar to compact bone (Aspden 1998). Harty (1957) proposed that the calcar acts as a 'spike' in certain fall situations, contributing to splitting of the trochanter. We hypothesised that among elderly fallers, the size of the calcar would influence whether fractures occurred in the trochanteric (TR) or femoral neck (FN) site. We also asked whether patients who sustained a fracture had more or less calcar bone than frailty-matched controls that fell but didn't fracture.
The FEMCO study is designed to investigate male (M) and female (F) patients with acute hip fracture with multi-detector CT, before they undergo surgery. It includes an age, sex and frailty-matched control group (who have sustained at least one injurious fall without hip fracture). The fractured hip is reconstructed in 3D for classification of fracture type (FN or TR). For the present pilot study, there were 14 cases (5TR, 9FN mean 80+/-8.5yrs. 7M, 7F) and 11 controls (83+/-7.0yrs. 3M, 8F). Axial CT slices where a calcar was visible were opened in Stradwin 4.1 software (Treece 2011). The calcar femorale was semi-automatically selected with the flood fill tool. Each axial image that contained a visible calcar was included in the analysis, so that for each femur a single calcar volume was generated. Results were examined using ANOVA.
Combining male and female results, there was a non-significant trend towards a higher calcar volume in patients sustaining trochanteric rather than femoral neck fractures (0.73cm3 +/- 0.26 vs 0.61cm3 +/-0.14, p=0.27) but no difference between cases and controls. Males had a significantly higher calcar volume than females (mean 0.82cm3 +/- 0.24 vs 0.59cm3 +/- 0.13, p=0.005). Further studies are now planned in larger samples of each sex, to examine the role of the calcar in fracture mechanics. Three-dimensional visualisations provide a novel insight into the damage patterns and resultant fragment locations.

P053
EFFECTS OF MYOSTATIN (GDF-8) ON MUSCLE AND BONE HEALING FOLLOWING DEEP PENETRANT MUSCULOSKELETAL INJURY
M Elkasrawy[1], D Immel[2], X Wen[3], X Liu[3],L Liang[4], MW Hamrick*[5]
[1]School of Dental Medicine, University of Colorado, USA; [2]Savannah River National Laboratory, USA; [3]Bioengineering Program, Clemson University, USA; [4]The Tauri Group, USA; [5]Georgia Health Sciences University, Medical College of Georgia, USA
Myostatin (GDF-8) is known to play an important role in muscle regeneration, and myostatin is also expressed during the early phases of fracture healing. In this study we used fluorescent immunohistochemistry to define the temporal and spatial localization of myostatin during muscle and bone repair following deep penetrant injury in a mouse model. We then used hydrogel delivery of exogenous myostatin in the same injury model to determine the effects of myostatin exposure on muscle and bone healing. Results show that while myostatin was constitutively expressed in the cytoplasm of intact skeletal muscle fibers, a pool of intense myostatin staining was observed amongst injured skeletal muscle fibers 12-24 hours post-surgery. Myostatin was also expressed in the soft callus chondrocytes 4 days following osteotomy. Hydrogel delivery of 10 or 100 ug/ml recombinant myostatin decreased fracture callus cartilage area relative to total callus area in a dose-dependent manner by 41% and 80% ($p<0.05$), respectively, compared to vehicle treatment. Myostatin treatment also dose-dependently decreased fracture callus total bone volume by 23% and 47% ($p<0.05$), with the higher dose of recombinant myostatin yielding the greatest decrease in callus bone volume. Finally, exogenous myostatin treatment caused a significant, dose-dependent increase in fibrous tissue formation in skeletal muscle. Together, these findings suggest that myostatin may inhibit bone repair after traumatic musculoskeletal injury through both autocrine (soft-callus chondrocytes) and paracrine (surrounding injured muscle fibers) mechanisms. Thus, early pharmacological inhibition of myostatin is likely to improve the regenerative potential of both muscle and bone following deep penetrant musculoskeletal injury.

P054
ESTIMATES OF CORTICAL THICKNESS FROM THE SUPERIOR FEMORAL NECK ARE A RISK MARKER FOR HIP FRACTURE IN WOMEN FROM CAMBRIDGE AND PRAGUE
KE Poole*[1], PM Mayhew[1], M Horak[2], J Reeve[1], JJ Stepan[2]
[1]NIHR Biomedical Research Centre, University of Cambridge, Cambridge, UK; [2]Charles University, Prague, Czech Republic
Introduction. Ageing is associated with progressive loss of bone in the superior femoral neck. Older individuals with better preservation of the superior cortex might be more resistant to hip fracture during falls, stumbles and trips. Among healthy elderly Icelandic men and women, a superior cortex thicker than 0.5mm (estimated with computed tomography (CT)) conferred significant protection against subsequent hip fracture (Johannesdottir ECTS 2010). With the present study, we investigated whether similar CT estimates of cortical thickness were a risk marker for hip fracture in women from Prague (Hip Joint in Trauma Study) and Cambridge (FEMCO study).
Methods. Female volunteers awaiting surgery for an acute hip fracture (n62) and matched controls without fracture (n62) consented to a multi-detector clinical CT scan of both hips (52 age-matched pairs from Prague and 10 pairs from Cambridge, median age 79 IQR 74-85, n32 cervical and n30 trochanteric fractures). Scans were performed lying on a calibration phantom. During CT image processing, the intact contra-lateral proximal femur was extracted from soft tissue. Mid femoral neck estimates of cortical thickness were made in four anatomical quadrants as described previously (Poole JBMR 2010). Using the Bone Investigational Toolkit (BIT2, Mindways software), estimates from 6 cross-sections (1mm apart) were combined into a single mean estimate for each quadrant (Superoanterior-SAQ, Inferoanterior-IAQ, Inferoposterior-IPQ and Superoposterior-SPQ). Exclusion criteria were malignancy, severe hip osteoarthritis and Paget's, osteomyelitis or metalwork in either hip. Differences between cases and controls were analysed by repeated measures MANOVA.
Results. Cortical bone in the superior quadrants appeared nearly twice as thick in controls compared with cases (e.g. median case SPQ 0.4mm IQR 0.3-0.6mm

vs control 0.8mm IQR 0.4-1.3mm), but there were no significant differences in the inferior quadrants (whole model p=0.0068, between-quadrant contrast, p=0.008). Using Mindways reference data, only 12/62 fracture cases and 3 controls had an areal BMD T score <-2.5 at the femoral neck. Johannesdottir's proposed cortical threshold of 0.5mm in SAQ would identify 26/62 fracture cases and 9 controls.
Conclusion. In agreement with Johannesdottirs' study and using similar clinical CT technology, these results confirm the association of reduced superior cortical thickness with hip fracture.

P055
AGE-DEPENDENT CHANGES IN OSTEOCLAST FORMATION IN A NEW STRAIN OF P2X7 RECEPTOR KNOCKOUT MICE
A Agrawal*[1], S Syberg[2], NR Jørgensen[2], A Gartland[1]
[1] Academic Unit of Bone Biology, The Mellanby Centre for Bone Research, Department of Human Metabolism, The University of Sheffield, Beech Hill Road, Sheffield, S10 2RX, UK; [2]University Hospital of Copenhagen Glostrup , Research Center for Ageing and Osteoporosis and Dept of Clinical Chemistry, Glostrup Ndr Ringvej 57-59, DK-2600 Glostrup, Denmark
The P2X7 receptor (P2X7R) is an ion-gated channel belonging to the family of purinergic receptors that get activated by extracellular nucleotides. The role of the P2X7R in regulating osteoclastogenesis has been described using knockout mice as well as primary human osteoclast cultures. These studies have contradictory findings as blocking the receptor in vitro prevented fusion of human monocytes to form multinucleated osteoclasts, whilst two different models of knockout mice retained the ability to form multinucleated osteoclasts in vivo and in vitro. The mice used in these studies have a natural mutation in the gene coding for P2X7R leading to reduced receptor function. Therefore, we generated mice with no known mutation in the P2X7R to better determine its role in the process of osteoclastogenesis. Spleens from age-matched WT and KO BALB/c mice were removed and the mononuclear fraction isolated. Equal numbers of cells were seeded onto glass coverslips or dentine discs and cultured in the presence of M-CSF and RANKL. Cells on glass coverslips were cultured for 7 days and TRAP stained to assess the number of multinucleated osteoclasts. Dentine discs were stained after 9 days to assess resorption. KO mice had a significantly higher number of multinucleated, TRAP positive cells and increased resorption compared to WT controls at 3 months of age. Mature, 6 month old, KO mice had a higher number of multinucleated TRAP positive cells, however, the resorption was the same in KO and WT cultures. These data suggest that receptor deletion has a role in enhancing osteoclast formation and subsequently function in young and developing mice. In older mice whilst there was an increase in osteoclast formation and activity in both WT and KO mice compared to 3 months of age, the higher number of osteoclasts observed in KO mice did not lead to a significantly higher amount of resorption compared to WT. We therefore propose the P2X7-/- BALB/c mice as a new model for studying the role of P2X7 receptor and that P2X7R in these mice enhances the differentiation and survival of osteoclasts but reduces their resorptive activity with increasing age.

P056
SINGLE LEG SQUAT PERFORMANCE IN ANTERIOR CRUCIATE LIGAMENT INJURED SUBJECTS
PA Rimmer*, PE Roos, K Button, V Sparkes, RWM Van Deursen
School of Heathcare Studies, Cardiff University, Cardiff, UK
Evidence suggests that anterior cruciate ligament (ACL) injured individuals do not use the same movement strategies as healthy individuals. It is unknown how this may affect them in more challenging activities of daily living and sport. The aim of this study is to evaluate how ACL injured patients perform a single leg squat (SLS) compared to healthy controls. SLS was evaluated as it is more challenging than gait and therefore more relevant to clinical decision making about progressing to sporting maneuvers.
To date, 6 ACL deficient (ACLD) (5 males, 1 female; mass=88±22 kg; height=1.78±0.11 m; age=35±11 years), 5 ACL reconstructed (ACLR) (5 males; mass= 83±12 kg; height=1.74±0.07 m; age=29±10 years) and 5 controls (3 males, 2 females; mass= 72±13 kg; height=1.70±0.09 m; age=30±3 years) performed a SLS on the injured leg for the ACL injured participants and the dominant leg for the control group. Motion analysis was performed using a Vicon Nexus system and a Kistler force platform. Knee extension moments and angles were calculated using Vicon Nexus software.
The ACLD group had reduced peak flexion angles compared to ACLR and control groups (65±5, 77±7 and 82±9 degrees respectively). Peak extension moments were similar across all groups (ACLD= 0.94±0.26 Nm/kg, ACLR=1.06±0.37 Nm/kg, control=1.04±0.36 Nm/kg). Peak knee moments occurred just after peak flexion and therefore at a smaller flexion angle for the ACLD group compared to the ACLR and control group (59±13, 75±7 and 80±6 degrees). Extension moments were similar when evaluated at a consistent angle of 50 degrees (ACLD=0.70±0.30Nm/kg, ACLR=0.63±0.34Nm/kg control=0.61±0.32Nm/kg).
In this sample, the controls squatted deepest followed by the ACLR group, with the ACLD group squatting least deep. This did not translate to an identical pattern for the knee extensor moments. Performance of ACL injured individuals needs to be evaluated on more challenging tasks to fully assess recovery. Further research, with more subjects, will clarify if ACLD individuals are using a strategy to protect their knee or if others factors are preventing them from squatting deeper. This would suggest that these individuals may not have fully recovered and will not be able to perform more challenging activities

P057
FEAR OF RE-INJURY IMPACTS REHABILITATION OF ACL INJURED PATIENTS
PE Roos*, K Button, PA Rimmer, RWM van Deursen
School of Healthcare Studies, Cardiff University, Cardiff, UK
ACL injured patients show variability in the ability to perform functional activities (Button et al., 2006). It is unknown whether this is due to differences in physical capability or whether fear of re-injury plays a role. Fear of re-injury is not commonly addressed in rehabilitation. This study aimed to investigate whether fear of re-injury impacts rehabilitation of ACL injured patients.
An initial group of five ACL reconstructed participants (ACLR, age: 30±11 years, weight: 815±115 N, height: 1.74±0.07 m, all male), five ACL deficient participants (ACLD, age: 31±12 years, weight: 833±227 N, height: 1.80±0.11 m, four male and one female), and five healthy controls (age: 30±3 years, weight: 704±126 N, height: 1.70±0.09 m, three male and two female) were compared. Fear of re-injury was assessed using the Tampa Scale for Kinesiophobia (Kvist, 2004). Quadriceps strength was measured on a Biodex dynamometer. Functional activity was assessed by a single legged maximum distance hop (on the injured leg for ACL patients). Motion analysis was performed with a VICON system, and a Kistler force plate. Hop distance was calculated using the ankle position. The peak knee extension moment during landing, and the knee angle at this peak moment were calculated in VICON Nexus.
The ACLD group scored worse on the Tampa scale for Kinesiophobia than the ACLR group (32±4 and 26±4). The ACLD patients did not hop as far as the ACLR and control groups (1.0±0.3, 1.3±0.1 and 1.4±0.3 m). The peak knee extension moments during landing were lowest in the ACLD group (263±159 Nm), slightly higher in the control group (354±122 Nm) and highest in the ACLR group (490±222 Nm), while knee flexion angles at these moments were similar (ACLD: 28±11, ACLR: 33±7 and control: 36±13 degrees). The ACLD group had weaker quadriceps than the control group, while the ACLR group was stronger (143±44 Nm, 152±42, and 167±50 Nm respectively).
Fear of re-injury and decreased quadriceps strength potentially both impact on the functional performance of ACL injured patients. Rehabilitation of ACL injured patients could therefore be improved by addressing strength and fear of re-injury. Future research with more participants will further clarify this.

P058
EFFECT OF METAL ION COMBINATIONS ON OSTEOBLASTS IN VITRO
K Shah*[1,2], M Wilkinson[1,2], A Gartland[1]
[1]Mellanby Centre for Bone Research, Department of Human Metabolism, University of Sheffield, UK; [2]NIHR Bone Biomedical Research Unit, Northern General Hospital, Sheffield, UK
Bone related adverse events including failure of implant osseo-integration, periprosthetic fracture, femoral neck narrowing, and unexplained pain occur more frequently following metal-on-metal hip resurfacing (MoMHR) versus total hip arthroplasty (THA). The exact mechanism for the adverse effects is still unclear and may be due to the direct effect on bone cells of metal ions released from the prostheses.
The aim of the present study was to determine the effect of clinically relevant combinations of metal ions on osteoblast cell survival and function. To assess cell proliferation and alkaline phosphatase (ALP) activity of osteoblasts, human osteoblast cells (SaOS-2), were cultured in 96-well plates for 24-hours and then treated with metal ions. Cell proliferation was measured at day 3 and day 7 using MTS assay, whilst ALP activity was assessed at day 3 by measuring pNPP substrate hydrolysis by the cell lysate. Mineralisation ability of the cells was assessed in 24-well plates cultured until day 21 and staining the calcium deposits using Alizarin red. All cultures were treated with the IC50 concentration of Co(II) (135µM) and an equivalent Cr(III) concentration (1Co(II):1Cr(III)).
After 3 days, Co(II) at an IC50 concentration decreased osteoblast proliferation as expected, but no further decrease in proliferation was observed with the 1Co(II):1Cr(III) combination treatment. However, after 7 days, a further significant decrease ($P<0.05$) in proliferation was observed with the combination treatment compared to Co(II) IC50. A similar significant decrease ($P<0.01$) was observed for ALP activity at day 3 with 1Co(II):1Cr(III) compared to Co(II) alone. For mineralization, a significant reduction ($P<0.0001$) was observed for Co(II) IC50 concentration, however no further reduction was seen with the 1Co(II):1Cr(III) combination treatment.
The observed decrease in cell proliferation and ALP activity with combination treatments suggest an additive detrimental effect compared to single ions alone. The mineralisation ability did not show any additive effect due to cell toxicity of chronic exposure to IC50 concentrations calculated from 3 day proliferation cultures. The results suggest that presence of both cobalt and chromium ions in the periprosthetic environment have more severe detrimental effect on

osteoblasts than single ions alone and extend our understanding of the periprosthetic bone health.

P059

PREVENTING INFECTION ON ANTIMICROBIAL SPACERS

BDW Richards*, R Bayston, W Ashraff
Division of Orthopaedic and Accident Surgery,University of Nottingham, Nottingham, UK

Background; Antibiotic loaded bone cement spacers are used as an adjunct to treatment in 2-stage arthroplasty revisions. If release of the correct choice of antimicrobials is optimised, systemic therapy might be curtailed and emergence of resistance minimised. Aims: To determine the elution period of antimicrobials from bone cement with and without a copolymer, polyvinylpyrrolidone (PVP) and to limit resistance development by the use of two or more antimicrobials.

Methods: Triclosan, gentamicin and clindamycin with and without (PVP) in CMW bone cement, was tested against six bacteria using serial plate transfer.

Results: While there was little difference between clindamycin and clindamycin with PVP, and between gentamicin and gentamicin with PVP, there was marked enhancement of release of triclosan with PVP. Resistance developed when antimicrobials were used singly but not when used in combination.

Conclusion: The addition of water soluble PVP was expected to enhance elution of antimicrobials from bone cement. This occurred with triclosan, a poorly water-soluble agent, but there was no significant difference for gentamicin and clindamycin, which as preferentially water -soluble. Other copolymers are being explored in an attempt to enhance their release. Triclosan used in combination extended the duration of activity against the test bacteria without development of resistance. Combinations of antimicrobials reduce the risk of paradoxical resistance in bone cement.

P060

A POLYMORPHISM IN THE INHIBIN ALPHA-SUBUNIT GENE IS ASSOCIATED WITH BONE MINERAL DENSITY (BMD) AND SERUM INHIBIN B (INH B) CONCENTRATIONS IN MEN

S Sim*[1], I Fogelman[2], G Hampson[1,2]
[1]Department of Clinical Chemistry, St Thomas' Hospital, London UK; [2]Osteoporosis Clinic, Rheumatology, Guy's Hospital, London, UK

Osteoporosis is a common complex polygenic disorder. However, the genes identified so far explain only a small proportion of the total genetic variation. New pathways may be implicated, particularly in men. The endocrine pathway involving the inhibins may be of importance. The inhibin alpha-subunit (INH-alpha) gene is proposed as a candidate gene because of its role in a feedback loop with sex hormones and gonadotrophins and its direct effect on bone remodelling. The aim of the study was to investigate the possible association between a polymorphic variant in the INH-alpha gene with BMD and circulating inh B in men.

We studied 146 men aged mean [SD] 57 [13.7] years, 54 with osteoporosis and 92 controls. The polymorphism -16C>T in the 5'UTR of INH-alpha gene was screened in all subjects. BMD was measured at lumbar spine (LS), femoral neck (FN) and total hip (TH). Serum concentrations of FSH, LH, testosterone, inh B were determined.

The prevalence of CC was 60%, CT: 37% and TT: 3%. After correction for age, height, weight and risk factors such as smoking habits, alcohol intake, exercise, dietary calcium intake , previous fracture, we found a significant independent association between INH-alpha genotype and BMD at the LS (p= 0.042). Z-score at the LS was lower in subjects with 'CT' and 'TT' genotype compared to 'CC' (mean [SEM] ; CT/TT : - 1.03 [0.17], CC : -0.5[0.21] p=0.08). In a multi-linear regression model, which included the gonadotrophins and serum testosterone, INH-alpha genotype was found to be independently associated with serum inh B (p= 0.043). Subjects with the 'CC' genotype had higher serum inh B concentrations compared to 'CT' and 'TT' genotypes (mean [SEM]; CC: 129.8 [9.34], CT/TT: 106 [8.4] pg/ml, p=0.06).

These preliminary data suggest that INH-alpha gene and the endocrine pathway involving the gonadal peptides, inhibin A and B, may be involved in the pathogenesis of osteoporosis in men. Further larger studies are needed for confirmation.

P061

ANALYSIS OF FAILED VAN STRATEN LPM PIP PROSTHESES

MC Bone[1]*, JL Cunningham[2], J Field[3], TJ Joyce[1]
[1]School of Mechanical and Systems Engineering, Newcastle University, UK; [2]Department of Mechanical Engineering, University of Bath, UK; [3]Cheltenham General Hospital, Cheltenham, UK

Finger arthroplasty lacks the success seen with hip and knee joint replacements. The Van Straten Leuwen Poeschmann Metal (LPM) prosthesis was intended for the proximal interphalangeal (PIP) joints. However revision rates of 30% after 19 months were reported alongside massive osteolysis. Three failed LPM titanium niobium (TiNb) coated cobalt chrome (CoCr) components were obtained- two distal and one proximal.

All three components were analysed using an environmental scanning electron microscope (ESEM). This gave the chemical composition of the surface to determine if the TiNb surface coating was still intact. The distal components were analysed using a ZYGO non-contact profilometer (1nm resolution) with the proximal component unable to be analysed due to its shape. ZYGO analysis gave the roughness average (Ra) of the surface and determined the presence of scratches, pitting and other damage.

Images obtained from both the ZYGO and the ESEM indicated that the surfaces of all components were heavily worn. On the articulating surfaces of both distal components unidirectional scratching was dominant, while the non-articulating surface showed multidirectional scratching. The presence of unidirectional scratching suggested two-body wear, whilst the multidirectional scratching on the non-articulating surface of the distal component suggested that trapped debris may have caused three-body wear.

The ESEM chemical analysis showed that in some regions on the distal component the TiNb coating had been removed completely and in other areas it had been scratched or penetrated. On the proximal component the TiNb coating had been almost completely removed from the articulating surfaces and was only present in small amounts on the non-articulating surfaces. There was little evidence of bone attachment to the titanium coating which was intended to help provide fixation.

ESEM images showed the coating had been removed in some sections where there was minimal scratching, suggesting this scratching did not impact significantly in the coating removal. Therefore here the main cause of coating removal may have been corrosion, although scratching may have also have played a part.

The osteolysis reported clinically may have been linked to the wear debris from the failed coating.

P062

INTERLAMELLAR INTERACTIONS IN FINITE ELEMENT ANALYSIS OF THE INTERVERTEBRAL DISC

BJ Luxmoore*, VN Wijayathunga, S Rehman, RK Wilcox
Institute of Medical and Biological Engineering, Department of Mechanical Engineering, University of Leeds, Leeds, UK

The annulus fibrosus (AF) of the intervertebral disc (IVD) has a unique, complex structure. If engineered tissues for the IVD are to be successfully developed, it is essential that the constituent level mechanics of the tissues in their natural form are fully understood (Nerurkar, J. Biomech. 2010).

Published finite element (FE) models of the IVD do not represent lamellae behaviour and are validated using bulk mechanics of the intervertebral joint. This study aims to develop models of the IVD that include representation of the lamellae structure of the AF and the behaviour of this tissue within the disc.

Three FE models of a vertebra-disc-vertebra section were developed considering the following scenarios of the AF:

1. Homogenous AF.
2. Concentric rings representing AF's lamellae structure with frictionless contact between rings.
3. Concentric rings with 'interface' elements representing the interlamellar space; properties were derived through calibration of a separate model of an AF tissue sample with histological studies of the AF (Gregory, J. Biomechs. 2009).

Displacements, stiffness and disc bulge were compared with the literature.

The properties derived for the interface elements were stiffer than those for the AF tissue. this is in agreement with in vitro studies that have examined the mechanisms by which the lamellae fail prior to the interlamellar interaction (Veres, Spine, 2010).

The macro-scale performance of the disc was sensitive to how the interlamellar interactions were modelled. Disc stiffness reduced by 7.1% between the homogenous and frictionless models. Use of the interface model improved the agreement with the in vitro performance of the disc: 5.8% error was recorded for disc stiffness and 2.1% error for disc bulge.

The mechanics of the lamellae within the AF changed significantly between the frictionless and interface models. The relative displacement of adjacent lamellae was reduced by 15% between the frictionless and interface models.

This study shows that the representation of the lamina structure of the AF affects the mechanics of the whole disc. Discrepancies in the modelling of interlamellar mechanics could have a significant effect on the interpretation of several important aspects of the biomechanics of the IVD.

P063

EXPRESSION OF CALCIUM SENSING RECEPTOR AND RECEPTOR ACTIVITY MODIFYING PROTEINS IN CELLS INVOLVED IN CALCIUM HOMEOSTASIS AND FLUORESCENCE RESONANCE ENERGY TRANSFER BASED STOICHIOMETRIC ANALYSIS OF THEIR INTERACTIONS

AJ Desai*, DJ Roberts, GO Richards, TM Skerry
Mellanby Centre for Bone Research, Department of Human Metabolism, University of Sheffield, UK

The G-protein coupled receptor known as the Calcium Sensing Receptor (CaSR) is known to play an important role in calcium homeostasis by sensing changes in extracellular calcium and modulating secretion of calcitropic hormones. A recent study revealed that cell surface expression of the CaSR

requires its association with single transmembrane accessory protein called Receptor Activity Modifying Protein (RAMP), specifically either RAMP1 or 3.
We hypothesize that efficiency of interaction and co-localization of RAMPs with CaSR could be determined using stoichiometric Fluorescence Resonance Energy Transfer (FRET) analysis.
In transfected COS7 cells, CaSR+RAMP1/3 complexes are highly co-localized around the peri-nuclear region indicative of co-localization in the endoplasmic reticulum and the Golgi bodies and also in regions of the cell membrane. The CaSR+RAMP3 FRET complex is ~25% more efficient than CaSR+RAMP1 FRET complex. Furthermore, the fraction of RAMP3 involved in FRET is ~18.8% more than RAMP1 whereas the fraction of CaSR involved in FRET with RAMP3 is ~33% more than with RAMP1.
To determine whether RAMP interactions with CaSR in cells involved in calcium homeostasis had roles beyond trafficking, we explored their expression in thyroid and bone cell lines.
We detected the expression of mRNA and protein for CaSR, RAMP1 and 2 in thyroid medullary carcinoma cell line (TT cells) using quantitative PCR, immunocytochemistry and western blotting. However, we could not detect expression of CaSR and RAMP3 mRNA transcripts in pre-osteoblastic MG63, SAOS-2 and TE85 cell lines using quantitative PCR. A high dose of extracellular calcium and differentiation of these cells into mature osteoblasts did not induce expression of CaSR or RAMP3. This suggests that osteosarcoma cell lines are not ideal models of osteoblasts to study CaSR expression and function.
The novel finding of the current study is that it provides a stoichiometric insight into CaSR-RAMP interaction and also indicates that CaSR interacts more efficiently with RAMP3 efficiently than RAMP1. This data is a step forward from the existing knowledge of CaSR-RAMP interaction. The increased understanding of their interaction will clarify their role in physiology and patho-physiology.

P064
EFFECT OF INVERSION AND EVERSION OF THE FOOT AT THE SHOE-PEDAL INTERFACE ON QUADRICEPS MUSCLE ACTIVITY, KNEE ANGLE AND KNEE DISPLACEMENT IN CYCLING
A Berry, N Phillips, V Sparkes*
School of Healthcare Studies, Cardiff University, Cardiff, UK
Knee injuries in cyclists are often thought to result from an imbalance of load during the cycling motion as a consequence of inappropriate bike set-up. Recently, it has been postulated that incorrect foot positioning may be a significant factor in lower limb injury and poor cycling performance. The purpose of this study is to assess the effect of changing the foot position at the shoe-pedal interface on Vastus Medialis (VM) and Vastus Lateralis (VL) activity (mean and mean peak), knee angle and knee displacement.
Maximum power tests were completed on a first visit, with data collection on a second visit recorded at 60% of the subjects maximum. Video footage and surface electromyography (SEMG) from VM and VL muscles was obtained. Data was recorded over 10 crank cycles in 3 experimental conditions; neutral, 10 degrees inversion and 10 degrees eversion using Ethylene Vinyl Acetate (EVA) wedges fitted between the cyclists shoe and the shoe cleat. Raw data (mean SEMG, mean peak SEMG) was obtained using Noraxon and SiliconCOACH measured knee angle and knee displacement. Data was analyzed using Friedmans test with appropriate post hoc tests.
12 male subjects (range 26-45, mean 35.9 years) completed the study. Mean and mean peak SEMG data showed no significant differences between the 3 experimental conditions for VM and VL. VM:VL ratios from raw mean SEMG data demonstrated a decrease in synchronicity in inversion and eversion compared to neutral. Pronators demonstrated most synchronicity in inversion and least synchronicity in eversion. There were statistically significant differences in knee angle and knee displacement between neutral, inversion and eversion ($p<0.05$). Inversion promoted smaller knee valgus angles and greater knee displacement from the bike. Eversion promoted larger knee valgus angles and a smaller displacement from the bike.
By altering the foot position to either 10 degrees inversion or 10 degrees eversion, knee angle and knee displacement can be significantly influenced. Clinically, subjects who foot type is classified as pronating may benefit from some degree of forefoot inversion posting. Further research on subjects with knee pain needs to be undertaken.

P065
INTRAOSSEOUS TRANSCUTANEOUS AMPUTATION PROSTHESIS: AN IN VITRO INVESTIGATION OF THE EFFECT OF FIBROBLAST PRE-SEEDING ON KERATINOCYTE ATTACHMENT TO TITANIUM ALLOY
CJ Pendegrass[1], O Shastri*[1], GW Blunn[1]
[1]The Institute of Orthopaedics & Musculoskeletal Science, Centre for Biomedical Engineering, University College London, UK
BACKGROUND: Transcutaneous implants have been successfully employed in dentistry since the 1960s; however attempts to translate this into extra-oral solutions for amputees have been beset with complications, with infection being the primary failure modality. Intraosseous transcutaneous amputation prostheses (ITAP) aim to overcome this problem sealing the skin-implant interface, preventing bacterial invasion and providing secure attachment of artificial limbs for amputees. Dermal fibroblasts and epidermal keratinocytes are known to effect one another's differentiation and cellular activities, such as basement membrane constitution. Cells do not adhere directly to biomaterial substrate, but attach to the surface via a layer of ECM extruded from cells. In keratinocytes this layer is principally laminin-5 (Ln5). Pre-seeding ITAP biomaterials with fibroblasts could up-regulate Ln5 expression and result in improved adherence of keratinocytes. This may improve the infection-resistance of the skin-ITAP seal in vivo. We hypothesise that keratinocyte adhesion, measured by the number of focal adhesions per unit cell area, will be significantly greater on titanium alloy substrates pre-seeded with fibroblasts compared with controls. METHODS: 25,000 PNT-2 keratinocytes in 70 microlitres were cultured on 10mm diameter Ti6Al4V discs. Discs were divided into 2 groups: one with a pre-seeded layer of 30,000 1BR3 fibroblasts 24h previously, and one without fibroblasts. Cells were fixed at 4 and 24h timepoints and immunolocalisation performed. Ln5 deposition was semi-quantitatively analysed for Order and Intensity. Vinculin was assessed by number of focal adhesions per unit cell area. RESULTS: Order and Intensity of Ln5 deposition were significantly greater in co-culture (PNT-2+1BR3) compared with PNT-2 alone ($p<0.05$). The median vinculin count per unit cell area at both 4 and 24hrs was significantly greater in co-culture (PNT-2+1BR3) compared with PNT-2 only ($p<0.05$). CONCLUSION: This study shows that the presence of fibroblasts in co-culture significantly increases keratinocyte adhesion in vitro. It also demonstrates that in co-culture, keratinocytes not only secrete more Ln5, but also show an increased order of deposition compared with those in mono-culture. Factors responsible may be direct, with integrin mediated attachment, or indirect; via fibroblast ECM or exogenous factors. This phenomenon may prove beneficial for tissue engineering approaches at improving the skin-implant seal around ITAP.

P066
VITAMIN D STATUS AND ITS CORRELATION WITH BLOOD PRESSURE AND PLASMA RENIN LEVEL IN PRE-MENOPAUSAL SAUDI WOMEN: A CROSS-SECTIONAL STUDY
SH AlMazidi*, H AlKadi, M Ardawi
Saudi Arabia
Vitamin D deficiency is one of major health problems with high prevalence in Saudi Arabia. Hypertension is another common health problem in the adult Saudi population. An association between blood pressure levels and vitamin D status had been suggested in several clinical and epidemiological studies. This relation could be secondary to the effect of vitamin D on calcium homeostasis. However, experimental work later on showed that vitamin D inhibits renin gene expression. This is due to receptors for 1,25(OH)2D3 have been found in many tissues including the cardiovascular system. Findings indicate that 1,25(OH)2D3 deficiency may play a role in the pathogenesis of hypertension. To our knowledge, there are no studies on the relationships between blood pressure levels and vitamin D status in the Saudi population.
To correlate vitamin D levels and blood pressure in relation to renin hormone in Saudi premenopausal women..
A cross-sectional study was conducted in 2010 at king Fahad research center-Jeddah, kingdom of Saudi Arabia included 201 healthy adult Saudi premenopausal females (20-45 years old). Blood pressure was measured using an automated blood pressure monitor (BPTru) that has been validated by the British Hypertension Society (BHS). Blood sample was obtained from each participant after 10 minutes of rest and centrifuged at 2500g for 10 min within 30 minutes of sample collection. Serum was stored at - 80 degrees C until analyzed. Serum colicalciferol, parathyroid and renin hormones measured by sandwich chemiluminescence immunoassay method.
Vitamin D showed that 34% were sever deficient (< 12.5 nmol/L) , 41% were moderate deficient (12.5 - 25 nmol/L), 23% were mild deficient (> or equal to 50 - < or equal to 75 nmol/L), and 2% were insufficient (> or equal to 25 - < 50 nmol/L). None of the studied subjects were sufficient (>75 nmol/L). All subjects included in the study were normotensive.
Correlation studies showed that there was a negative correlation between vitamin D level and both systolic and diastolic blood pressure.

P067
A LIMPING CHILD, A RARE DIAGNOSIS, A REMARKABLE RECOVERY
E Koushiappi*, R Abbas, E Mughal
New Cross Hospital, Wolverhampton, UK
We report of an unusual case of acute infective adductor myositis in a healthy child, following trauma to the muscles of the medial thigh compartment.
A 9 year old boy presented with four days of limping and progressive right hip pain, associated with high grade pyrexia. A history of groin trauma was reported. He has no past medical history. The child was unable to weight-bear, had right groin tenderness and very limited active right hip movements. Passive right hip movements were painful and restricted, particularly internal rotation and adduction. Blood results showed a Raised Erythrocyte Sedimentation rate (ESR), C-Reactive Protein (CRP) and Creatine Protein Kinase (CPK), with normal White Cell Count (WCC). Ultrasound excluded septic arthritis and did not reveal any other pathology.
The child remained unwell despite broad-spectrum intravenous antibiotics and adequate analgesia. Magnetic resonance imaging demonstrated florid

abnormality in right obturator externus, adductor brevis and adductor magnus muscle, but no definite well-formed abscess. Blood cultures revealed Staphylococcus aureus. He was treated with intravenous narrow-spectrum antibiotics for five days and then switched to oral antibiotic therapy. He was followed-up with weekly bloods and made a very good recovery.
Classically, infective myositis has been a tropical disease. The incidence has been increasing worldwide due to Human Immunodeficiency Virus (HIV) emergence and immunosuppression.
This is a rare presentation as the boy was not immunocompromised, and the trauma sustained may be implicated in the pathogenesis. Nonetheless, it is likely that the trauma was coincidental with the disease process. Furthermore, to our knowledge, no case has previously reported post-traumatic bacterial myositis in this muscle group.
Limping due to hip pain is a common complaint in children. The diagnosis-making process depends on many factors, including the age of the child and clinical presentation. This case illustrates the importance of early magnetic resonance imaging in patients that remain unwell, despite excluding septic arthritis. Moreover, it may support the evidence of trauma involvement in the disease aetiology and progression.

P068
THE EFFECTIVENESS OF CERVICAL SPINE ORTHOSES AT RESTRICTING SPINAL MOTION: A 3-D MOTION ANALYSIS STUDY
N Evans*[1], G Hooper[2], R Edwards[2], G Whatling[3], V Sparkes[3], C Holt[3], S Ahuja[2]
[1]Cardiff School of Engineering, Cardiff University, UK; [2]Cardiff Spinal Unit, University Hospital of Wales, Cardiff, UK; [3]Cardiff School of Healthcare Studies, Cardiff University, UK
Assessing the efficacy of cervical orthoses in restricting spinal motion has historically proved challenging due to a poor understanding of spinal kinematics and the difficulty in accurately measuring spinal motion. This study is the first to use an 8 camera optoelectronic, passive marker, motion analysis system with a novel marker protocol to compare the effectiveness of the Aspen, Aspen Vista, Philadelphia, Miami-J and Miami-J Advanced collars. Restriction of cervical spine motion was assessed for physiological and functional range of motion (ROM).
Nineteen healthy volunteers (12 female, 7 male) were fitted with collars by an approved physiotherapist. ProReflex (Qualisys, Sweden) infra-red cameras were used to track the movement of retro-reflective marker clusters attached to the head and trunk. 3-D kinematic data was collected from uncollared and collared subjects during forward flexion, extension, lateral bending and axial rotation for physiological ROM and during five activities of daily living (ADLs). ROM in the three clinical planes was analysed using the Qualisys Track Manager (Qualisys, Sweden) 6 Degree of Freedom calculation to determine head orientation relative to the trunk.
For physiological ROM, the Aspen and Philadelphia were more effective at restricting flexion/extension than the Vista ($p<0.001$), Miami-J ($p<0.001$ and $p<0.01$) and Miami-J Advanced ($p<0.01$ and $p<0.05$). The Aspen was more effective at restricting rotation compared to the Vista ($p<0.001$) and Miami-J ($p<0.05$). The Vista was least effective at restricting lateral bending ($p<0.001$). Through functional ROM, the Vista was less effective than the Aspen ($p<0.001$) and other collars ($p<0.01$) at restricting flexion/extension. The Aspen and Miami-J Advanced were more effective at restricting rotation than the Vista ($p<0.01$ and $p<0.05$) and Miami-J ($p<0.05$). All the collars were comparable when restricting lateral bending.
The Aspen is superior to, and the Aspen Vista inferior to, the other collars at restricting cervical spine motion through physiological ROM. Functional ROM observed during ADLs are less than those observed through physiological ROM. The Aspen Vista is inferior to the other collars at restricting motion through functional ROM. The Aspen collar again performs well, particularly at restricting rotation, but is otherwise comparable to the other collars at restricting motion through functional ranges.

P069
PUTTING THE SPRING IN YOUR STEP - A NOVEL CRUTCH DESIGN
W Rudge*, R Weiler, P Smitham, C Holloway, N Papadosifos, J Maswania, S Grange
University College London, UK
Modern forearm crutches have evolved little since their invention last century. We evaluated comfort and user satisfaction of 2 spring-loaded crutches compared with existing crutch designs.
25 healthy subjects (11 male, average age 26.2 years; 14 female, average age 22.7 years) participated. Each used 5 different crutches in a randomly allocated order:
i) standard forearm crutch (ergonomic grip);
ii) spring-loaded crutch (soft spring, ergonomic grip);
iii) spring-loaded crutch (firm spring, ergonomic grip);
iv) standard forearm crutch (normal grip);
v) axillary crutch.
Participants completed a purpose built course at the Pedestrian Accessibility and Movement LAboratory, UCL (PAMELA). The course consisted of a mixture of slopes (transverse and longitudinal), sprint, slalom, and a slow straight. All participants completed questionnaires relating to crutch user preference and design features.
Crutches were ranked in order of preference. The crutch least favoured was the axillary design, irrespective of subject weight, followed by the standard forearm crutch with normal grip. The 3 crutches with ergonomic handles all scored similarly. Preferences were also analysed in two weight controlled groups and compared against the soft and firm spring-loaded crutches. Of the lighter group 80% preferred the softer spring. Of the heavier group 56% preferred the firmer spring. Over 50% of subjects rated handle/cuff comfort as a key feature in crutch design.
Preference for different spring tensions depended on subject weight, which should be the focus of further research. The least favoured crutches were the axillary and standard issue forearm grip crutch. Comfort was the most important feature in crutch design with preference for ergonomic handles, followed by cuff design ranked the most important. Spring-loaded crutches performed comparably to the other crutches with ergonomic handles.

P070
Abstract withdrawn

P071
Abstract withdrawn

P072
EVALUATION OF THE ACCURACY OF THE I-BUTTON FOR THE MEASURING PATIENT COMPLIANCE WITH BRACE WEAR IN IDIOPATHIC CLUB FOOT
PN Unnikrishnan*[1], VK Peter[1], HL George[3], J Sampath[4]
[1]Department of Trauma and Orthopaedics, Alder Hey Childrens Hospital (Royal Liverpool Childrens), UK
Club foot or Congenital Talipes Equino Varus deformity is a congenital condition affecting one in every 1,000 live births. The main stay in the treatment of this condition is repeated manipulation and casting followed by bracing (Ponsetti technique) for a period of up to 2 years to maintain the corrected position.
The most important causes for failure after successful correction using the Ponseti technique is failure of compliance with brace wear. It is extremely difficult to monitor the duration of brace wear in the patient's home environment. The aim of the study was to evaluate the accuracy of using the Thermocon I- Button as a method for objective assessment of compliance with brace use.
The study was a prospective method comparison study commenced after obtaining ethical approval. Children who presented to the orthopaedic departments at our tertiary children's hospital with clubfeet and considered suitable for treatment by the Ponseti method were invited to participate. There were no specific exclusion criteria. The I -Button was used for collecting the data of time and temperature. The sensor was recessed within the insole. A rise in temperature of greater than 3 degrees was considered significant. The time the brace was applied and removed was also manually recorded using a stopwatch by one observer. The data was assessed for the limits of agreement using the Bland Altman Plot.
There were 30 patients. 14 cases were bilateral and 16 unilateral. Of those 9 patients had their right side affected and the other 7 it was the left. The difference between the two sets of data is likely to differ by less than 8 minutes and this deviation could be in either direction. The 95% confidence intervals are -6.64 to 8.15. The agreement between the two sets of data was found to be statistically significant.
This study proves that the temperature sensor can accurately identify when the brace is in use and when it is taken off. The I -Button temperature sensor therefore can be used to accurately assess the period of brace wear and hence this device can be used to evaluate patient compliance with brace wear.

P073
CALCIFIC TENDINITIS OF QAUDRICEPS AND PATELLAR TENDONS AFFECTING THE SAME KNEE SIMULTANEOUSLY
R Abbas*[1], K Bitar[1], T Malik[2], B Ahmed[2], R Koka[2]
[1]Department of Trauma and Orthopaedics, New Cross Hospital, UK; [2]Department of Orthopaedics, Eastbourne Hospital, UK
We report an unusual case of knee disease where calcific tendonitis occurring in both quadriceps and patellar tendon simultaneously in the same knee. A 47 year old female presented to orthopaedics outpatient clinic with acute onset of swelling and knee pain with no history of trauma. She was found to have a moderate effusion of the knee joint with mild tenderness over the mid quadriceps tendon. Active flexion of the knee joint was painful with a range of motion between 0-90 degrees. She is otherwise healthy with no past medical history. Plain radiographs and Magnetic Resonance Imaging (MRI) Scan revealed calcification of both tendons.
Calcific tendonitis is classically found in the supraspinatus tendon of the shoulder. In addition, it has been described in other areas of the body such as the wrist, thigh, hip, knee and ankle. This condition usually occurs in the quadriceps or patellar tendons separately and rarely affecting both tendons in the same knee

simultaneously. The patients condition improved significantly with physiotherapy, anti-inflammatory medications and ultrasound therapy.
Calcific tendinitis of both quadriceps and patellar tendon is a very rare cause of knee pain. Most of the time it is treated conservatively with non-steroidal anti-inflammatory drugs and ultrasound therapy and some times steroid injection. However; patient may require surgical intervention especially in refractory cases to resolve the condition.

P074
Abstract withdrawn

P075
BONE MICROSTRUCTURE AND FRACTURE PREDISPOSITION IN YOUNG RACEHORSES
CL Nicholson*[1], EC Firth[1], MR Waterland[2], G Jones[2], S Ganesh[2], RB Stewart[3]
[1]Institute of Veterinary, Animal & Biomedical Sciences, [2]Institute of Fundamental Sciences, [3]Institute of Natural Resources, Massey University, New Zealand
Musculoskeletal injury is a major reason for loss of performance in the equine athlete. Morphological changes in mineralised tissue have been observed at the exact sites of fracture initiation in young Thoroughbred horses before racing or training has even begun. This suggests that there may be factors related to early bone and joint development that contribute to later musculoskeletal problems and that some individuals could be inherently predisposed to fracture. The aim of this study was to determine whether differences in the chemical structure and composition (microstructure) of bone correlate with fracture predisposition.
Small ex vivo bone sections were taken from the third metatarsal bone of nine horses at two specific sites associated with morphological abnormalities and at one control site. The animals consisted of four newborn and four 5-month-old foals, all of which were apparently normal, and one 3-year-old horse with known morphological abnormalities. Sections were dehydrated in ethanol and embedded in polymethylmethacrylate before the sample surface was carefully polished for subsequent microstructural investigation by Fourier transform infrared microspectroscopy (FTIR) in reflectance mode. Multiple FTIR spectra were collected in both the calcified cartilage and subchondral bone of each section. Discriminant analysis of the spectra was used to determine whether there were differences between individual horses or between normal and abnormal bone.
Striking microstructural variations were observed in the apparently normal 5-month-old foals, raising intriguing questions around whether these differences simply represent normal variations between individuals or if such variations during early growth could have implications for bone health in later life. Discriminant analysis of samples from all nine animals revealed three distinct groupings related to age and health, with the first two discriminant functions separating newborns from older foals and apparently normal animals from the abnormal horse.
While microstructural differences appear to exist amongst individuals and between animals of different ages and disease status, further work is required to establish the key features of the bone mineral and collagen that distinguish normal bone development from those which may compromise fracture resistance.

P076
INVESTIGATION OF 3D PLANAR WRIST MOVEMENTS DURING ACTIVITIES OF DAILY LIVING
A Gilmour*[1], J Richards[2], DRM Redfern[3]
[1]Wishaw General Hospital, NHS Lanarkshire, UK; [2]School of Sport, Tourism & the Outdoors, University of Central Lancashire, UK; [3]Department of Orthopaedics, Lancashire Teaching Hospitals NHS Trust, UK
Several authors have used 3D motion analysis to measure upper limb kinematics, but none have focused solely on wrist movements, in six degrees of freedom, during activities of daily living (ADL). This study aimed to determine the role of the different planar wrist movements during three standardised tasks, which may be affected by surgical procedures.
Nine volunteers (age range 22-45) were recruited and each participant performed three simulated ADLs: using a door lever, a door knob and opening/closing a jam jar. The ADLs were simulated using a work-sim kit on an isokinetic dynamometer. Motion analysis was performed by a 10-camera Oqus system (Qualisys Medical AB, Gothenburg, Sweden). All raw kinematic data were exported to Visual3D (C-Motion Inc.), where the biomechanical model was defined and joint kinematics calculated.
Table 1 shows a similar range of radial-ulnar deviation and flexion-extension as previous studies. However a substantial amount of wrist rotation also occurred in all tasks. This was significantly greater when using the door lever compared with the door knob and jam jar tasks.
Previous studies have stated that a negligible degree of rotation occurs at the wrist. This study found a maximum mean of 31.7 degrees of wrist rotation. This indicates that considerable rotational movement occurs at the wrist during certain functional tasks. Surgical approaches and clinical pathology may disrupt structures responsible for rotational stability. Further investigation of this rotational component of carpal movement during additional ADLs is proposed in both normal and clinical subjects, to explore the potential relationship between carpal surgery and rotational laxity.

P077
COMPARISON BETWEEN APTUS AND DVR PLATE AS THE PREFERRED METHOD OF DISTAL RADIUS FRACTURE FIXATION
R Singhal*[1], M Shakeel[1], S Dheerendra[1], P Ralte[2], S Morapudi[1], M Waseem[1]
[1]Department of Orthopaedics, Macclesfield District General Hospital, UK,[2]Department of Orthopaedics, Royal Liverpool Hospital, UK
Background: Volar locking plates have revolutionised the treatment for distal radius fractures. The DVR (Depuy) plate was one of the earliest locking plates which were used and they provided fixed angle fixation. Recently, newer volar locking plates, such as the Aptus (Medartis), have been introduced to the market that allow the placement of independent distal subchondral variable-angle locking screws to better achieve targeted fracture fixation. The aim of our study was to compare the outcomes of DVR and Aptus volar locking plates in the treatment of distal radial fractures.
Methods: Details of patients who had undergone open reduction and internal fixation of distal radii from October 2007 to September 2010 were retrieved from theatre records. 60 patients who had undergone stabilisation of distal radius fractures with either DVR (n=30) or Aptus (n=30) plate were included in the study.
Results: Mean age of patients undergoing fixation using DVR plate was 56.6 years (n=30) with 22 females and 8 males. Fractures in this group included 20 type 23-C, three type 23-B and seven type 23-A. The patients were followed up for an average of 5.5 months (2-16 months). 3 patients underwent revision of fixation due to malunion (n=1), non-union (n=1) and failure of fixation (n=1). Four patients had reduced movements even after intensive physiotherapy necessitating removal of plate.
Mean age of patients undergoing Aptus volar locking plate fixation was 56.38 years (n=30) with 21 females and 9 males. There were 27 type 23-C, two type 23-B and one type 23-A fractures according to AO classification. The patients were followed up for an average of 4.1 months (2-11 months). 2 patients developed complex regional pain syndrome (CRPS) and 1 patient underwent removal of screws due to late penetration of screws into the joint.
Conclusion: Complex and unstable fractures of the distal radius can be optimally managed with volar locking plates. Both systems are user friendly. Aptus plates provide an additional advantage of flexibility in implant positioning and enhanced intra-fragmentary fixation compared to the DVR plate. In our study Aptus plates had lower secondary surgical procedures compared to DVR plates.

P078
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P079
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P080
MICROARRAY ANALYSIS OF THE EFFECT OF MILD HYPOTHERMIA ON PRIMARY RAT OSTEOBLASTS
JJ Patel*[1], D Hyliands[2], D Talbot [2], F-L Lim[2], DJ Mazzatti[2], TR Arnett [1]
[1]Department of Cell and Developmental Biology, University College London, London, UK; [2]Unilever Discover, Unilever PLC, Sharnbrook, Bedfordshire, UK
Core body temperature tends to decline with age due to reductions in heat production and peripheral vasoconstriction. Core temperatures of 35.5C or below are common in the elderly. We have found that hypothermia (34C - 35.5C) inhibits in vitro bone formation by osteoblasts, whilst stimulating osteoclast formation. In this study, we have investigated further the effects of mild hypothermia (35.5C) on osteoblast function using gene arrays. Primary osteoblasts were obtained from neonatal rat calvaria by trypsin/collagenase digestion. Cells were cultured at 35.5C and 37C for up to 14 days in medium (DMEM) supplemented with beta-glycerophosphate, ascorbate and dexamethasone. RNA samples were analysed at days 7 and 14, representing early and late osteoblast differentiation, respectively, using Agilent whole genome arrays. Analysis of day 7 cultures identified 192 down-regulated and 90 up-regulated transcripts at 35.5C, relative to 37C control. Down-regulated genes included PAK1 and transcripts involved in the NF-kappa-B pathway, and PLOD2 and transcripts involved in the TGF-beta-1 pathway; both pathways are reported to promote osteoblast proliferation and differentiation. Up-regulated transcripts included FGF 21, over-expression of which in mice is associated with a fall in core body temperature, greater sensitivity to insulin, and resistance to diet-induced obesity. At day 14 however, 274 transcripts were up-regulated and only 77 were down-regulated at 35.5C. In contrast to day 7, transcripts included in the NF-kappa-B and TGF-beta-1 pathways were up-regulated. Klotho, which is required for normal bone formation in mice, and may slow ageing, was also up-regulated. The shift in gene expression patterns between days 7 and 14 is consistent with the observed effects of chronic hypothermia on osteoblast function (strong initial inhibition, followed by recovery). These data suggest that hypothermia exerts complex actions on osteoblast function.

P081

SINGLE RADUIS OF CURVATURE IMPLANT DESIGN ENHANCES POWER OUTPUT FOLLOWING TOTAL KNEE ARTHROPLASTY

D Hamilton*[1,2], P Gaston[2], AHRW Simpson[1,2]
[1]Orthopaedic Engineering Collaboration, University of Edinburgh, UK; [2]Royal Infirmary of Edinburgh, UK

End-stage osteoarthritis is characterised by pain and reduced physical function, for which total knee arthroplasty (TKA) is recognised to be a highly effective treatment. Most implants are multi radius in design, though modern kinematic theory suggests a single flexion / extension axis is located in the femur. A recently launched TKA implant (Triathlon, Stryker US), is based on this theory, adopting a single radius of curvature femoral component. It is hypothesised that this design allows better function, and specifically, that it results in enhanced efficiency of the quadriceps group through a longer patello-femoral moment arm.

Change in power output was compared between single and multi radius implants as part of a larger ongoing randomised controlled trial to benchmark the new implant. Power output was assessed using a Leg Extensor Power Rig, well validated for use with this population, pre-operatively and at 6, 26 and 52 weeks post-operatively in 101 Triathlon and 82 Kinemax implants. All patients were diagnosed with osteoarthritis, and drawn from a single centre. Output was reported as maximal wattage (W) generated in a single leg extension, and expressed as a proportion of the contralateral limb power output to act as an internal control.

The results are shown in the table below. Two-way repeated measures ANOVA demonstrated a significant effect of TKA on the quadriceps power output, $F = 249.09$, $p = <0.001$ and also a significant interaction of the implant group on the output $F = 11.33$, $p = 0.001$. Independent samples t-tests of between group differences at the four assessment periods highlighted greater improvement in the single radius TKA group at all post-operative assessments ($p <0.03$), see table.

The theoretical enhanced quadriceps efficiency conferred by single radius design was found in this study. Power output was significantly greater at all post-operative assessments in the single radius compared to the multi radius group. This difference was particularly relevant at early 6 week and 1 year assessment. Lower limb power output is known to link positively to functional ability. The results support the hypothesis that TKAs with a single radius design have enhanced recovery and better function.

P082

EXPERIMENTAL GLUCOCORTICOID INDUCED OSTEOPOROSIS: DIFFERENCES BETWEEN MOUSE STRAINS

Y Vattakuzhi*, AR Clark, NJ Horwood
Kennedy Institute of Rheumatology, Imperial College Faculty of Medicine, London W6 8LH, UK

Glucocorticoids (GCs) are frequently used in inflammatory diseases like rheumatoid arthritis, asthma and inflammatory bowel disease. Long term use of GCs, however, can lead to the development of GC induced osteoporosis (GIO). GIO is characterised by increased osteoclast (OCL) activity and decreased osteoblast activity leading to bone loss; paradoxically, GCs are also reported to inhibit p38 MAPK activity and to directly inhibit OCL function. Experimental models of GIO are well established in the outbred Swiss Webster mice and show loss of bone volume and trabecular connectivity. However, most genetically modified mice are on C57BL/6 background and the aim of this study was to induce GIO in this strain.

CD1 mice were used instead of Swiss Webster mice in these experiments; they are an outbred strain similarly derived from the original Swiss mice. Bone marrow cells of C57BL/6 and CD1 mice were used to investigate the effect of GCs on OCL formation and activity in vitro and to assess p38 activity. To determine the in vivo effects of GCs in the two mouse strains, slow release pellets containing the GC prednisolone were implanted subcutaneously into C57BL/6 and CD1 mice to induce GIO. 3.2mg/kg/day of prednisolone was released over a 58 day period. Lumbar vertebrae and tibiae were then analysed by bone histomorphometry and micro CT analysis. Bone marrow cells were harvested for colony forming assays to determine differences in the progenitor cell populations after GC treatment as well as for in vitro OCL assays.

GCs inhibited p38 activity after RANKL stimulation in C57BL/6 mice; however, in vitro OCL formation and activity was not affected. Long term treatment with prednisolone did not reduce trabaecular volume and bone mineral density compared to placebo in C57BL/6 mice in contrast to CD1 mice. In addition no differences were observed in progenitor cell populations in C57BL/6 mice.

Experimental models of GIO are therefore dependent on the genetic background of the mice with CD1 mice being more susceptible than C57BL/6 mice to glucocorticoid induced bone loss.

P083

RISK FACTORS OF OSTEOPOROSIS IN POSTMENOPAUSAL ALBANIAN WOMEN

A Kollcaku*, A Tafaj, J Kollcaku, E Petrela
University Hospital Center, Tirana, Albania

Objective of this study was to evaluate relationship between bone mineral density (BMD) changes and risk factors in osteoporosis.

Method: This cohort prospective study included 507 eligible women. Bone mass measurement was performed by Quantitative Ultrasound System at the heal bone (calcaneus). A detailed questionnaire for determination of risk factors for osteoporosis (number of children born; menopause age, coffee and tea consumption, smoking, corticosteroids, rheumatic diseases, body mass index, lifestyle etc) was administered to all subjects enrolled in this study.

Results: Prevalence of Osteoporosis was 4.73%. Important statistical relationships were found by using Kendal correlation coefficient between menopause and BMD changes ($r = 0{,}174$; $p=0{,}001$), and also between BMD changes and body mass index (BMI) ($r=0{,}111$; $p=0{,}003$). Through multiple regression analysis were found important relationships between BMD changes (dependent variable) and number of children born ($p=0{,}003$), coffee consumption ($p=0{,}048$), treatment with diuretics ($p=0{,}050$), rheumatoid arthritis ($p=0{,}035$).

Conclusions: responsible factors for changes on BMD in Albanian post menopause women, except menopause, are coffee consumption, BMI, number of children born, treatment with diuretics and rheumatoid arthritis.

P084

MINIMALLY INVASIVE OXFORD PHASE 3 UNICOMPARTMENTAL KNEE REPLACEMENT: THE FIRST 48 CASES IN SHANGHAI, CHINA

YH Tu*[1], HM Xue[1], XD Liu[1], MW Cai[1], ZD Xia[2], D Murray[2]
[1] Department of Orthopaedics, Yangpu District Central Hospital, 450 TengYue Road, Shanghai, 200090 China. [2]Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Oxford University, NOC, Headington, Oxford OX3 7LD

Great interest in unicompartmental knee arthroplasty (UKA) for medial osteoarthritis has rapidly increased following the introduction of minimally invasive UKA (MI-UKA). This approach preserves the normal anatomy of knee, causes less damage to extensor mechanism and results in a more rapid post-operative recovery. However, experience with this approach is limited in China. The aim of this report was to determine the short-term clinical and radiographic outcomes of MI-UKA in the Chinese, and to identify any features that are unique to this population. Fifty two knees, in forty-eight patients, with medial compartmental osteoarthritis treated by MI-UKA via C-arm intensifier guide (CAIG) from May 2005 to January 2009 were reviewed. Pain and range of motion (ROM) was assessed using the HSS scoring system before and after surgery. Pre- and postoperative alignment of the lower limbs was measured and compared. The mean follow up time was 24 months (12-42 months). In all cases the pain over medial compartment of the knees was relieved or subsided. The post-operative ROM was 0-136 degree (mean 122degree) , and the mean alignment was 2degree varus (0- 7degree varus). The HSS score increased from 72(61-82) to 92(72-95). 93% of the postoperative scores were good or excellent. Interestingly, the distribution of femoral component sizes of these patients was XS 2%, Small 83%, Medium 15%, Large 0%, XL 0%; whereas tibial component size was AA 27%, A 55%, B 15%, C 3%, D 0%, E 0%, and F 0%. The optimal fitted match between tibial and femoral size was: tibia AA and A with XS and small femur, tibia B and C with medium femur. The estimated match was: tibia D and E with large femur, tibia F with XL femur. In contrast to the Oxford report, the sizes of these components are smaller and not in correlation with the height, weight and BMI of the patients. We conclude that MI-UKA is an effective method for treating medial compartmental osteoarthritis of the knee in the Chinese population. CAIG is a feasibly intraoperative measure to predict femoral component sizes. However, component sizes and combinations are different from the Oxford guideline.

P085

LACK OF EFFECT OF ADENOSINE ON RODENT OSTEOBLASTS AND OSTEOCLASTS

MOR Hajjawi*, IR Orriss, TR Arnett
Department of Cell and Developmental Biology, University College London, UK

Adenosine is a nucleoside present within cells. In the extracellular environment it is found at concentrations from 30-300nM, rising to 10 micromolar in some pathological settings. It has important roles as an extracellular signalling molecule in many cell and tissue types, acting through P1 receptors. Although ATP signalling in bone cells (through P2 receptors) is now well documented, the potential osteotropic actions of adenosine, a key hydrolysis product of ATP, are less well understood. The aim of this study was to determine the effect of exogenous adenosine on 1) the growth, differentiation and bone-forming ability of osteoblasts; 2) the formation and resorptive function of osteoclasts. Primary osteoblasts were obtained from neonatal rat calvariae by trypsin/collagenase digestion and cultured for up to 14 days with 1nM-100micromolar adenosine or the synthetic agonist, 2-chloroadenosine. Mineralised bone nodule formation was assessed by image analysis of alizarin red stained cell layers. RT-PCR was used to study the expression of P1 receptors at 4, 7 and 14 days of culture and cell number was determined colorimetrically. Osteoclasts, formed from the bone marrow of 6 week old mice, were cultured on ivory slices with 200ng/ml M-CSF and 3ng/ml RANKL. Adenosine was either added throughout or for the final two days of culture when the medium was acidified to pH 6.9 to activate

resorption. Adenosine had no effect on osteoblast cell number at any stage. The formation of mineralised 'trabecular-shaped' bone structures was also unaffected. Similar effects were observed with 2-chloroadenosine. RT-PCR demonstrated that the adenosine A1 receptor was expressed at a very low level on osteoblasts, whilst no expression of the A2a or A2b receptors was detected. Rodent heart and lung were used as a PCR positive control. Continuous adenosine treatment had no effect on osteoclast formation or resorptive activity. A single dose of adenosine to mature osteoclasts was also without effect. Although these experiments do not exclude the possibility that the cultures already contained saturating concentrations of endogenous adenosine, our results suggest that adenosine, in contrast with ATP, may be unlikely to function as a major local signalling molecule for bone cells.

P086

THE TIMING OF HIGH TIBIAL OSTEOTOMY IN RELATION TO AUTOLOGOUS CHONDROCYTE TRANSPLANTATION IN VARUS GONARTHROSIS OF THE KNEE

NE Picardo*[1], ZS Nawaz[2], K Gallagher[3], P Whittingham-Jones[4], T Parratt[5], TW Briggs[6], R Carrington[7], JA Skinner[8], G Bentley[9]
Royal National Orthopaedic Hospital, Stanmore, UK

The aim of this study was to determine whether the clinical outcome of autologous chondrocyte transplantation was dependent on the timing of a high tibial osteotomy in tibio-femoral mal-aligned knees. Between 2000 and 2005, forty-eight patients underwent autologous chondrocyte implantation with HTO performed at varying times relative to the second stage autologous chondrocyte implantation procedure. 24 patients had HTO performed simultaneously with their second stage cartilage transplantation, (the HTO Simultaneous Group). 5 patients had HTO prior to their cartilage procedure, (the HTO pre-ACI Group) and 19 had HTO performed between 1 to 4 years after their second stage cartilage implantation, (the HTO post-ACI Group). There were 29 men and 19 women with a mean age of 37 years (Range 28 to 50) at the time of their second stage procedure.

With average follow-up of 72 months we have demonstrated a significant functional benefit in performing the HTO either prior to or simultaneously with the ACI procedure in the mal-aligned knee. The failure rate in the Post-ACI group was 45% compared to the Pre-ACI and Simultaneous group, with failure rates of 20% and 25%, respectively.

An HTO performed prior to or simultaneously with an autologous chondrocyte implantation procedure in the mal-aligned knee, provides a significant protective effect by reducing the failure rate by approximately 50%.

P087

Abstract withdrawn

P088

ASSOCIATIONS BETWEEN VITAMIN D2 AND D3 AND CORTICAL BONE PHENOTYPES IN CHILDHOOD: A PROSPECTIVE COHORT STUDY

A Sayers*[1], WD Fraser[2], DA Lawlor[3], JH Tobias[1]
[1]Muscloskeletal Research Unit, University of Bristol, UK; [2]Department of Muscloskeletal Biology, University of Liverpool,UK; [3]MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, UK

Introduction: Severe vitamin D deficiency, which is generally caused by reduced dietary intake and/or sun exposure, may lead to the clinical condition of osteomalacia/rickets as a result of defective skeletal mineralisation. However less is known about the consequences of milder vitamin D deficiency (insufficiency) for bone development in childhood.

Methods: Using 3260 children from the Avon Longitudinal Study of Parents and Children we examined the relationship between plasma levels of 25(OH)D2 and 25(OH)D3 measured at the median age of 9.9 years, and cortical bone geometry as measured at 50% mid tibia using pQCT at a median age of 15.5 years. Analyses, which were by bootstrap linear regression, were adjusted for age, sex, body composition, social economic position and physical activity. 25(OH)D3 was seasonally adjusted. Analyses between 25(OH)D3 and cortical bone outcomes were adjusted for 25(OH)D2 and vice versa.

Results: 25(OH)D2 was positively related to periosteal circumference (PC) [b=0.032 (95%CI: 0.003,0.062) p=0.03], but inversely related to cortical bone mineral density (BMDC) [b=-0.041 (95%CI: -0.073,-0.008) p=0.01] (b ~represents SD change per doubling of vitamin 25(OH)D2 or 25(OH)D3). 25(OH)D3 was unrelated to either of these parameters, but was instead positively related to cortical thickness, reflected by an inverse association with endosteal circumference adjusted for PC [b=-0.024 (95%CI: -0.040,-0.008) p=0.004].

Differing associations of 25(OH)D2 and 25(OH)D3 with cortical bone geometry were mirrored by distinct relationships with indices of bone strength: 25(OH)D2 was associated with reduced resistance to buckling, reflected by a positive association with buckling ratio (BR) [b=0.052 (0.008,0.097) p = 0.02]; in contrast, 25(OH)D3 was associated with greater resistance to buckling, shown by a negative association with BR [b=-031 (95%CI: -0.059,-0.004), p = 0.03]. These associations of 25(OH)D2 and 25(OH)D3 with BR differed statistically (p=0.001).

Conclusions: 25(OH)D2 and 25(OH)D3 levels as measured at a median of age 9.9 years had distinct relationships with cortical bone geometry as measured at age 15.5 years, and showed opposing associations with cortical bone strength. Although our results were adjusted for a range of possible confounders, residual confounding could still be responsible, or alternatively our results could reflect distinct biological actions of 25(OH)D2/D3 on cortical bone development.

P089

VALIDATION OF INERTIAL MOTION SENSORS (IMU) TO MEASURE SAGITTAL KNEE ANGLES DURING NORMAL GAIT AS COMPARED TO A GAIT LAB

MK Monda*[1], I McCarthy[1,2], M Thornton[1,2], P Smitham[2], AJ Goldberg[1,2]
[1] Institute of Orthopaedics & Musculoskeletal Science, University College of London, UK; [2]Royal National Orthopaedic Hospital NHS Trust, Stanmore, UK.

Knowledge of knee kinetics and kinematics contributes to our understanding of the patho-mechanics of knee pathology and rehabilitation and a mobile system for use in the clinic is desirable.

We set out to assess validity and reliability of ambulatory Inertial Motion Unit (IMU) Sensors (Pegasus®) against an established optoelectronic system (CODA®).

Pegasus® uses inertial sensors placed on subjects' thighs and lower leg segments to directly measure orientation of these segments with respect to gravity. CODA®) models the position of joint centres based on tracked positions of optical markers placed on a subject, providing 3D kinematics of the subject's hips, knees and ankles in all three planes.

Intra observer reliability of the Pegasus® system was tested on 6 volunteers (4 male; 2 female) with no previous lower limb or knee pathology. IMU's were placed on the long axis of the lateral aspects of both thighs and lower leg segments. A test re-test protocol was used with sagittal data angle collected around a standard circuit.

Inter-observer reliability was tested by placement of IMU's by 5 different testers on a single volunteer.

To test validity, we collected simultaneous sagittal knee angle data from Pegasus® and CODA® in two subjects. The presence of IMU's did not compromise positioning of optical markers.

Analysis of triplicate measurements showed that intra-observer error is +/- 5°. Inter-observer difference in measurements varied from 3° to 20° absolute values.

Positional error of the Pegasus® IMU's was significant in comparison to CODA®, with absolute offsets in knee angles typically of 10° to 25°. Range of motion differences between the two systems calculated as root mean square (rms) difference of the zero meaned signals were 3.8°-4.8°.

1. The Pegasus® system is useful in ambulatory measurement of the range of knee motion in the sagittal plane.
2. In the current configuration there was poor intra and inter-observer reliability possibly related to positional error using the Pegasus® system and may be due to fixation method, operator factors, body shape and variability of clothing.
3. Recommendations have been made to the manufacturer.

P090

THE DEVELOPMENT & VALIDATION OF A SOFTWARE TOOL (SHIPS) FOR MEASUREMENT OF OSTEOARTHRITIS-ASSOCIATED MORPHOLOGICAL PARAMETERS OF THE ACETABULUM & PROXIMAL FEMUR

S Thiagarajah*, SJ MacInnes, L Yang, JM Wilkinson
Bone Biomedical Research Unit, University of Sheffield, UK

Subtle variations in hip morphology associate with risk of hip osteoarthritis (OA). However, validated accurate methods to quantitate hip morphology using plain radiography are lacking. We have developed a Matlab-based software-tool (SHIPs) that measures 19 OA-associated morphological-parameters of the hip using a PACS pelvic radiograph. In this study we evaluated the accuracy and repeatability of the method.

Software accuracy was assessed by firstly measuring the linear ratio of 2 fixed distances and several angles against a gold-standard test radiograph, and secondly by repeated measurements on a simulated AP radiograph of the pelvis (reformatted from CT-data) that was digitally rotated about 3-axes to determine the error associated with pelvic mal-positioning. Repeatability was assessed using 30-AP Pelvic radiographs analysed twice (intra-observer), by 2 readers (inter-observer), and finally, using 2 pelvic radiographs taken in 23 subjects (n=46 radiographs) taken same day after re-positioning (short-term clinical-practice variability), and was expressed as coefficient of variation (CV%).

Software accuracy was 0.1% for linear measurements, and 0.2, 0.4, and 0.1 degrees, for angular measurements of 30, 60, and 90 degrees, respectively. Anterior rotation of the pelvis in the sagittal plane beyond 10 degrees produced a decrease in acetabular-tilt (-11 degrees at 20 degrees rotation) and acetabular-index-of-depth-to-width-ratio (-9.3% at 20 degrees rotation). Conversely, femoral-head-to-neck-ratio increased with both anterior and transverse rotation (+9% to +14% at 20 degrees rotation).

The intra-observer CV was between 0.3-6.3%, and inter-observer CV was between 0.7-14.9% for all measurements with the exception of the measurement of horizontal-toit-externa (HTE) that had intra and inter-observer CVs of 33.4

and 29.1%, respectively. Short-term clinical repeatability was between 0.4-8.5%, with the exception of HTE that was 20.7%).
This software showed good accuracy and precision for the measurement of OA-associated hip morphological-parameters from plain radiographs of the pelvis, and may be useful in clinical research studies quantitating the relationship of these parameters to the development of hip OA. The method is, however, sensitive to large variations in pelvic positioning and use of the HTE measurement is associated with poor repeatability that is likely due to poor definition of the bony landmarks used for this parameter.

P091
DIETARY CALCLIM INTAKE IN POST-MENOPAUSAL WOMEN IN THE METABOLIC BONE CLINIC: ASSESSMENT OF REQUIREMENT FOR CALCIUM-CONTAINING SUPPLEMENTS
A Chotai*[1], L Satkunananthan[1], R Inaoui[1], I Fogelman[1], G Hampson[1]
Osteoporosis Clinic, Rheumatology, Guy's Hospital, London, UK
Most guidelines recommend adequate calcium and vitamin D intake as part of prevention and treatment for osteoporosis. Calcium and vitamin D supplements are routinely prescribed as adjunct to bisphosphonate therapy. Concerns have been raised, recently, following reports of an increased risk of myocardial infarction in women given calcium supplementation. In contrast, epidemiological studies do not show increased cardiovascular risks with higher dietary calcium intake. In order to ascertain the need for calcium supplements, we assessed the dietary calcium intake of post-menopausal women attending the osteoporosis clinic and the prevalence of cardiovascular risk factors in this population. We studied 118 women aged mean [SD] 67[13] years. Seventy one (60%) were on bisphosphonates and 41 (34%) had a previous fragility fracture. Calcium intake was assessed by dietary recall on 2 consecutive days and calculated using a calcium intake calculator. The presence of cardiovascular risk factors which included ischaemic/cardiovascular disease (IHD/CVD), hypertension, hyperlipidaemia was recorded. The subjects were divided in quartiles based on their dietary calcium intake (Group 1 : 0-400 mg/day, Group 2 : 401-800 mg/day, Group 3 : 801-1200 mg/day, Group 4 : >1200 mg/day). Thirty eight women (32%) had a dietary calcium intake of greater than 800 mg/day (Group 3) and 41 (35%) had a dietary intake of greater than 1200 mg (Group 4). In Group 3, the number of patients with a history of IHD/CVD was 22 (58%), hypertension 15 (39%) and hyperlipidaemia 11 (29%). In Group 4, the numbers were as follows;IHD/CVD: 17 (41%), hypertension: 15 (37%), hyperlipidaemia: 5(12%). Many patients receiving bisphosphonates will be prescribed 1200 mg of calcium and 800 IU vitamin D. In view of recent concerns, reduced dosage of calcium supplements should be considered, depending on dietary calcium intake, particularly in the elderly.

P092
COMBINING MONOCYTES WITH CHONDROCYTES FOR THERAPEUTIC CARTILAGE REPAIR
J Wardale*[1], N Hopper[1], S Ghose[2], N Rushton[1]
[1]Orthopaedic Research Unit, University of Cambridge, Addenbrooke's Hospital UK; [2]Tigenix, Cambridge UK
Mesenchymal stem cells (MSCs) have potential for therapeutic repair of cartilage and bone but still require optimization in terms of their capacity to deposit an appropriate extracellular matrix (ECM). Adult human cartilage has a limited capacity for repair and is unusual in that it is one of the few tissues where injury is not followed by an influx of monocytes. We are studying the effects of co-culturing primary monocytes with MSCs differentiating along chondrogenic lineage but in addition we needed to investigate the effects of the monocytes on the mature chondrocytes that will result from the MSCs and will also be present in the host tissue.
Human articular cartilage chondrocytes were isolated from human donors undergoing knee replacement surgery for osteoarthritis (OA) with full ethical consent. Cultures were expanded and cells used below passage five for co-culture experiments. Monocytes were prepared from fresh heparinized human blood samples by Ficoll gradient. Co-cultures consisted of either chondrocyte micromasses overlaid with monocytes, or chondrocytes and monocytes seeded together within a collagen/glycosaminoglycan scaffold (Chondromimetic, Tigenix UK). Media, cell pellets and scaffolds were analysed for extracellular matrix (ECM) proteins and proteases by dot blot, western blot, zymography and immunohistochemistry.
Human chondrocytes maintained stable micromasses and laid down an ECM for at least 40 days. Human monocytes eventually formed a proliferating cell population with a rounded morphology on top of the chondrocyte micromasses. These cells established an adherent population with a fibroblastic morphology when replated on plastic. Analysis of chondrocyte ECM proteins indicated that monocytes affected deposition of types I and II collagen, decorin and fibronectin and the overall amounts of gelatinases released. RTPCR demonstrated a decrease in type I collagen expression and a concomitant increase in MMP13 expression.
The precise interaction between monocytes and and chondrocytes has yet to be established but is thought to involve a mixture of contact and paracrine factors. In this study co-culture of monocytes with chondrocytes resulted in phenotypic changes to the chondrocytes which may warrant the inclusion of monocytes in cartilage/bone repair and also provide information as to the responses of OA chondrocytes to external stimuli.

P093
DIRECT INHIBITION OF OSTEOCLAST FORMATION AND ACTIVITY BY THE VITAMIN E ISOMER GAMMA-TOCOTRIENOL
P Kalia*[1], R Brooks[1], DC Ireland[1], C Beeton[2], N Rushton[1]
[1]Orthopaedic Research Unit, Department of Surgery, University of Cambridge, UK; [2]Department of Medicine, University of Cambridge, UK
Introduction. Vitamin E homologues, specifically tocotrienols, have been shown to have favourable effects on bone and possess properties which suggest potential anti-resorptive activity. The hypothesis that tocopherols and/or tocotrienols might act as potential anti-resorptive agents was investigated by testing the effect of vitamin E compounds on osteoclast number, formation and resorption.
Materials and Methods. Human osteoclasts were cultured on collagen, dentine and calcium phosphate substrates with or without vitamin E homologues. Compounds were either added at the start of culture to study effects on osteoclast number and formation, and at the start of osteoclastic resorption to determine effects on activity.
Results and Discussion. With increasing dose of gamma-tocotrienol from 0.01 mM to 1 mM, MTS absorbance increased significantly (Figure 1, 0.01M - 0.14 ± 0.01 , 0.1mM - 0.24 ± 0.02 and 1mM - 0.67 ± 0.06; p=0.007). At 0.01 mM and 1 mM, delta-tocotrienol absorbance decreased significantly (0.05 ± 0.06 and 0, compared to 0.1 mM which had 0.17 ± 0.01; $p<0.05$). Comparing between the different treatment doses alpha-, delta- or gamma-tocotrienols (1 - 0.1 mM), significant differences were found depending on the dose (p=0.024, p=0.018 and p=0.007, respectively). Absorbance increased when the alpha-tocotrienol concentration was increased from 0.1 mM to 1 mM. Alpha- and gamma-tocotrienol inhibited TRAP+ osteoclast formation without reducing total cell number. A reduction in osteoclast-mediated resorption was observed after treatment with 1 and 0.1 mM delta-tocotrienol (Figure 2, 0.01 mM - 7.87 ± 3.68 %, 0.1 mM - 3.05 ± 1.20 %, 1.0 mM - 0.40 ± 0.80 %) and gamma-tocotrienol (0.01 mM - 2.60 ± 0.85 %, 0.1 mM - 2.37 ± 0.32 %, 1.0 mM - 0 %). Only gamma-tocotrienol inhibited osteoclast activity without consequent toxicity. These results were confirmed using cells from a second donor.
In summary, we have identified the vitamin E isomer gamma-tocotrienol as a non-toxic, anti-resorptive compound. Gamma-tocotrienol may have potential applications in the treatment of bone loss.

P094
PRECOCIOUS MATURATION OF ARTICULAR CARTILAGE: THE KEY TO CARTILAGE REPAIR AND REGENERATION?
IM Khan*[1], SL Evans[2], RD Young[3], EJ Blain[1], AJ Quantock[3], N Avery[4], CW Archer[1]
[1]School of Biosciences, Cardiff University; [2]School of Engineering, Cardiff University; [3]School of Optometry and Visual Sciences, Cardiff University; [4]Bristol Veterinary School, Bristol University, UK
One reason why NICE (National Institute for Clinical Excellence) does not support operations by the NHS to heal hyaline cartilage lesions using a patients own cells is because there is no clear evidence to show that these operations are beneficial and cost-effective in the long term. Specifically, NICE identified a deficiency of high quality cartilage being produced in repaired joints. The presence of high quality cartilage is linked to long-lasting and functional repair of cartilage. The benchmark for quality, NICE stipulate, is repair cartilage that is stiff and strong and looks similar to the normal tissue surrounding it, i.e. mature hyaline articular cartilage.
Biopsy material from autologous cartilage implantation surgical procedures has the appearance of immature articular cartilage and is frequently a mixture of hyaline and fibrocartilage. Osteoarthritic cartilage, in its early stages, also exhibits characteristics of immature articular cartilage in that it expresses proteins found in embryonic and foetal developmental stages, and is highly cellular as evidenced through the presence of chondrocyte clusters. Therefore, an ability to modulate the phenotype and the structure of the extracellular matrix of articular cartilage could positively affect the course of repair and regeneration of articular cartilage lesions. In order to do this, the biochemical stimuli that induce the transition of an essentially unstructured amorphous cartilage mass (immature articular cartilage) to one that is highly structured and ordered, and biomechanically adapted to its particular function (mature articular cartilage) has to be identified.
We show for the first time, that fibroblast growth factor-2 and transforming growth factor beta-1 induce precocious maturation of immature articular cartilage. Our data demonstrates that it is possible to significantly enhance maturation of cartilage tissue using growth factor stimulation; consequently this may have applications in transplantation therapy, or through phenotypic modulation of osteoarthritic chondrocytes in diseased cartilage in order to stimulate growth and maturation of repair tissue.

P095
DEVELOPMENT OF A COMPUTATIONAL MODEL FOR OPTIMISATION OF PERIPROSTHETIC FRACTURE FIXATION
JH Mak*[1], M Moazen[1], AC Jones[1], Z Jin[1], E Tsirdis[2,3,4], RK Wilcox[1]

[1]Institute of Medical and Biological Engineering, School of Mechanical Engineering, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT, UK; [2]Academic Department of Orthopaedic and Trauma, Section of Musculoskeletal Disease, Institute of Molecular Medicine, School of Medicine, University of Leeds, Woodhouse Lane, 6 Leeds, LS2 9JT, UK; [3]Department of Surgery and Cancer, Division of Surgery, Imperial College London, B-block Hammersmith Hospital, Du-Cane Road, London, W12 0HS;[4]Academic Orthopaedic Unit, Faculty of Medicine, Aristotle University of Thessaloniki, Thessaloniki, 54 124, Greece

Periprosthetic femoral fractures can occur as a complication of total hip arthroplasty and are often challenging to treat as the mechanical scenario is influenced by the presence of the metal prosthesis within the bone. This research focuses on finding the optimum fixation for transverse, Vancouver type B1 periprosthetic fractures, stabilised using locking plates and secured using screws. The aim of this study was to experimentally validate a computer model of a human femur, develop that model to represent a periprosthetic femoral fracture fixation and show how the model could be used to indicate differences between plating techniques.

In the first development stage, both a laboratory model and a finite element model were developed to evaluate the mechanical behaviour of an intact composite femur under axial loading. Axial strains were recorded along the medial length of the femur in both cases and compared to provide validation for the computational model predications. The computational intact femur model was then modified to include a cemented total hip replacement, and further adapted to include a periprosthetic fracture stabilised using a locking plate, with unicortical screws above, and bicortical screws below the transverse fracture.

For the intact femur case, the experimental and computational strain patterns correlated well with an average difference of 16%. Following the inclusion of the stem, there was a reduction in the strain in the region of the prosthesis reducing by an average of 45%. There was also a large increase in bulk stiffness with the introduction of the prosthesis. When the fracture and plate fixation were included, there was little difference in the proximal strain where the stem dominated, and the strains in the distal region were found to be highly sensitive to the distribution of the screws.

The results of this study indicate that screw configuration is an important factor in periprosthetic fracture fixation. A laboratory model of the periprosthetic facture case is now under development to further validate the computational models and the two approaches will then be used to determine optimum fixation methods for a range of clinical scenarios.

P096

A BOVINE CARTILAGE EXPLANT MODEL FOR THE STUDY OF STAPHYLOCOCCUS AUREUS INDUCED SEPTIC ARTHRITIS

IDM Smith*[1], KM Cyrulik[2], S Amyes[3], AHRW Simpson[1], AC Hall[2]
[1]Department of Trauma and Orthopaedics, The University of Edinburgh, UK; [2]Centre for Integrative Physiology, The University of Edinburgh, UK; [3]Department of Microbiology, The University of Edinburgh, UK

Staphylococcus aureus is the most common bacterial isolate in septic arthritis. From studies on isolated cartilage cells, the 'pore-forming' alpha and gamma toxins are considered the most virulent factors. However, understanding the response of in situ chondrocytes is important in order to identify new treatments to reduce the extent of cartilage damage during, and following, episodes of septic arthritis. Animal models can give useful information; however the interpretation of data can be complex because of the strong immune response. Thus, to clarify the role of S. aureus toxins on in situ chondrocytes we have developed a bovine cartilage explant model.

Metacarpophalangeal joints, from 3-year-old cows, were opened under sterile conditions within 6hrs of slaughter and cartilage explants harvested. Explants were placed into flasks containing Dulbecco's Modified Eagle Medium (DMEM). Aspirates from a patient with septic arthritis of the hip, containing S. aureus, were compared to negative aspirates (no bacterial growth) from a patient with an inflamed knee joint (controls).

The explants were incubated at 37 degrees Celsius and stained after 18, 24 and 40hrs with the fluorescent probes chloromethylfluorescein di-acetate and propidium iodide (10 micromolar each) to label living chondrocytes green and dead cells red respectively. Following imaging of cartilage by confocal laser scanning microscopy, the percentage cell death at each time point was obtained using Volocity 4 software.

There was no detectable change in chondrocyte viability (<1% cell death) over 40hrs incubation with the negative aspirate. However, for the aspirate from a patient positive for S. aureus, there was a rapid increase in cell death between 18 and 24hrs (0.2 +/- 0.3% to 23 +/- 5% cell death respectively) and almost complete cell death at 40hrs (80 +/- 12%; data are means +/- s.d; n=4).

These results show that a strain of S. aureus capable of manifesting clinical disease exerts a potent effect on in situ chondrocytes. In the absence of an immune response, chondrocyte death was purely the result of the bacteria and their products. This bovine cartilage explant model could therefore be useful for studying the effects of S. aureus on chondrocyte behaviour and, ultimately, cartilage integrity.

P097

PRELIMINARY REPORT OF MINIMALLY INVASIVE OXFORD PHASE 3 UNICONDYLAR KNEE ARTHROPLASTY VIA EXTRAMEDULLARY FEMORAL ALIGNMENT GUIDE FOR UNICOMPARTMENTAL KNEE OSTEOARTHRITIS

YH Tu*[1], HM Xue[1], XD Liu[1], MW Cai[1], ZD Xia[2]
[1]Department of Orthopaedics, Yangpu District Central Hospital, 450 Teng Yue Road, Shanghai, 200090 China; [2]Botnar Research Centre,Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Oxford University, NOC, Headington, Oxford OX3 7LD, UK

Intramedullary (IM) femoral alignment guide for unicondylar knee arthroplasty (UKA) is a classic and generally accepted technique to treat unicompartmental knee osteoarthritis.However,IM system has a risk of excessive blood loss,fat embolism and activation of coagulation.Moreover, the implant placement and limb alignment may be less accurate in IM for UKA than total knee arthroplasty.So we try to use extramedullary (EM) femoral alignment for UKA to avoid above disadvantages.To our knowledge,few current studies have been reported by now. We reported a series of cases treated through a newly developed EM technique and evaluated the accuracy of femoral component alignment and preliminary clinical results. Between January 2009 and January 2010, 11 consecutive patients(15 knees)consisting of 8 males and 3 females were enrolled.There were 7 cases in unilateral knee and 4 cases in bilateral knees.The mean age was 65.2 years (range 60~72 years).Incision,surgical time,blood loss and complications were measured.The pre- and post operative function of the knees were evaluated by HSS score system.The pre- and postoperative femoral component alignment was measured and compared. All cases were followed up for average 15 months (10-22 months). The mean length of incision was 7.2cm (range 6 to 8cm),the mean surgical time was 115.0min(range 90 to 125min),the mean blood loss was 50.8ml (range 50 to 80ml). The mean preoperative HSS score increased from 75 (range 63 to 83) to 95 (range 88 to 97) postoperatively ($p<0.05$). All femoral components were within the recommended range for varus/valgus (±10 degree) and lexion/extension (±5 degree) angle.None had complications associated with reamed canal injury.By using our EM technique,we could achieve an accurate femoral component alignment and satisfactory clinical effect.However,strict comparison between EM and cconventional IM technique and large amount of cases are essential.Further mid- and long-term studies are required.

P098

COULD EARLY FEMORAL NECK FRACTURE IN METAL-ON-METAL HIP RESURFACING BE ASSOCIATED WITH WEAR OF THE BEARING SURFACE?

JK Lord[1], DJ Langton[1], AVF Nargol[2], RMD Meek[3] TJ Joyce*[1]
[1]School of Mechanical & Systems Engineering, Newcastle University, UK; [2]Joint Replacement Unit, University Hospital of North Tees,Stockton, UK; [3]Southern General Hospital, Glasgow, UK

Metal-on-metal hip resurfacing prostheses are a relatively recent intervention for relieving the symptoms of common musculoskeletal diseases such as osteoarthritis. While some short term clinical studies have offered positive results, in a minority of cases there is a recognised issue of femoral fracture, which commonly occurs in the first few months following the operation. This problem has been explained by a surgeon's learning curve and notching of the femur but, to date, studies of explanted early fracture components have been limited.

Tribological analysis was carried out on fourteen retrieved femoral components of which twelve were revised after femoral fracture and two for avascular necrosis (AVN). Eight samples were Durom (Zimmer, Indiana, USA) devices and six were Articular Surface Replacements (ASR, DePuy, Leeds, United Kingdom). One AVN retrieval was a Durom, the other an ASR. The mean time to fracture was 3.4 months. The AVNs were retrieved after 16 months (Durom) and 38 months (ASR).

Volumetric wear rates were determined using a Mitutoyo Legex 322 co-ordinate measuring machine (scanning accuracy within 1 micron) and a bespoke computer program. The method was validated against gravimetric calculations for volumetric wear using a sample femoral head that was artificially worn in vitro. At 5mm3, 10mm3, and 15mm3 of material removal, the method was accurate to within 0.5mm3. Surface roughness data was collected using a Zygo NewView500 interferometer (resolution 1nm).

Mean wear rates of 17.74mm3/year were measured from the fracture components. Wear rates for the AVN retrievals were 0.43mm3/year and 3.45mm3/year. Mean roughness values of the fracture retrievals (PV = 0.754nm, RMS = 0.027nm) were similar to the AVNs (PV = 0.621nm, RMS = 0.030nm), though the AVNs had been in vivo for significantly longer.

Theoretical lubrication calculations were carried out which found that in both AVN retrievals and in seven of the twelve cases of femoral fracture the roughening was sufficient to change the lubrication regime from fluid film to mixed. Three of these surfaces were bordering on the boundary lubrication regime. The results show that even before the femoral fracture, wear rates and roughness values were high and the implants were performing poorly.

P099

DETERMINING THE 'RECRYSTALLISATION' ACTIVATION ENERGY OF BONE MINERAL

CE Greenwood*, KD Rogers, S Beckett
Cranfield Forensic Institute, Centre for Archaeological and Forensic Analysis, Department of Applied Science, Security and Resilience, Cranfield University, Shrivenham, Swindon, SN6 8LA, UK
The physico-chemical modifications to bone due to injuries such as heat treatment and diagenesis have been extensively researched in recent years. In particular, modifications to bone such as recrystallisation and thermal decomposition have been studied to enable species differentiation of fragmented bone material. This has proved beneficial within archaeological and forensic contexts. To date, the aforementioned modifications are assumed to be influenced by the relationship between the organic (collagen) and mineral (calcium hydroxyapatite) components of bone. This is largely because the complete decomposition of the organic component coincides with the recrystallisation of bone mineral (at temperatures ranging from 600 - 650oC). However, this relationship is not yet comprehensively understood and consequently the processes and mechanisms involved when bone mineral is heated are largely assumed and often ambiguous.
In order to begin to understand the relationship between the organic and mineral components of bone, there is a need to approach this from a fundamental level. A novel approach is currently being investigated where the extent of recrystallisation of bovine bone has been examined as a function of time and temperature. As a result, the activation energy for the recrystallisation of bovine bone mineral was found to be 89.5 kJmol-1. This has been compared to a collagen free nanoapatite. Contrasting these behaviours will lead to a better understanding of the mineral - organic relationship in bone.
The results of this research are anticipated to make a valuable contribution across a wide range of disciplines including archaeology, forensics and biomedicine.

P100
GINGIVAL SOFT TISSUE LIMITS ALVEOLAR BONE REGENERATION
MS Ghuman[1], FJ Hughes[1] and IJ McKay*[2]
[1]King's College London,Department of Periodontology, Dental Institute, Floor 18, Tower Wing, Guy's Hospital,London, UK; [2]Clinical and Diagnostic Oral Sciences, Barts and the London School of Medicine and Dentistry, Queen Mary, University of London, London, UK
Surgical treatment of periodontal disease to promote the regeneration of lost bone and tooth attachment often shows unpredictable outcomes. The presence of connective tissue during healing appears to be a negative regulator of bone regeneration. This study aimed to test the hypothesis that connective tissue can inhibit bone morphogenetic protein (BMP)-induced osteoblastic differentiation. The expression of putative inhibitors of BMPs in cultured rat gingival cells was determined using RT-qPCR. Constitutive expression of 7 BMP inhibitors was detectable in cultured gingival fibroblasts. Inhibitors showing the highest levels of basal expression were Gremlin 1, Nbl1 and Gremlin 2. In addition, Gremlin 1, Gremlin 2 and Nbl1 protein could all be detected by Western blotting. To test if any of the inhibitors are up-regulated by BMPs cultures of gingival and periodontal ligament fibroblasts were treated with 100ng/ml BMP-2 and expression measured after 2, 6 and 24 hours. In gingival fibroblasts changes in inhibitor mRNA expression was highly variable, but in periodontal ligament fibroblasts there was consistent and significant up-regulation of noggin expression. The in vivo expression of inhibitors was investigated by in situ hybridisation analysis of mouse mandibles that revealed expression of Gremlin-1 restricted to the inner half of the gingival lamina propria and the periodontal ligament. ROS 17/2.8 osteoblastic cells provide a sensitive system to measure BMP-activity. These cells show a consistent dose-response to BMP-2, up to 10ng/ml, which can be quantified by increased alkaline phosphatase activity. The BMP-inhibitory bioactivity of conditioned media (CM) from rat gingival fibroblasts was assessed against BMP-2 stimulated ROS cells. Conditioned media from gingival fibroblast showed significant inhibitory activity comparable to 100ng/ml noggin. The non-specific removal of Gremlin 1 using immunoprecipitation beads completely removed the inhibitory activity from gingival fibroblast CM. Inhibitory activity of depleted CM could be fully restored by the addition of recombinant Gremlin 1. These results suggest that these inhibitory molecules limit the clinical regeneration of alveolar bone following surgery and moreover it is mediated by BMP-inhibitors derived from the gingival connective tissue.

P101
HOW MANY DIFFERENT TYPES OF FEMORA ARE THERE IN PRIMARY HIP OSTEOARTHRITIS? AN ACTIVE SHAPE MODELLING STUDY
C Merle*[1], W Waldstein[1], JS Gregory[2], SR Goodyear[2], RM Aspden[2], PR Aldinger[3], DW Murray[1], HS Gill[1]
[1]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, UK; [2]Bone and Musculoskeletal Research Programme, Division of Applied Medicine, University of Aberdeen,UK; [3]Department of Orthopaedic Surgery, Paulinenhilfe, Diakonieklinikum Stuttgart, Germany
In uncemented total hip arthroplasty (THA), the optimal femoral component should allow both maximum cortical contact with proximal load transfer and accurate restoration of individual joint biomechanics. This is often compromised due to a high variability in proximal femoral anatomy. The aim of this on-going study is to assess the variation in proximal femoral canal shape and its association with geometric and anthropometric parameters in primary hip OA.
In a retrospective cohort study, AP-pelvis radiographs of 98 consecutive patients (42 males, 56 females, mean age 61 (range:45-74) years, BMI 27.4 (range:20.3-44.6) kg/m2) who underwent THA for primary hip OA were reviewed. All radiographs were calibrated and femoral offset (FO) and neck-shaft-angle (NSA) were measured using a validated custom programme. Point-based active shape modelling (ASM) was performed to assess the shape of the inner cortex of the proximal femoral meta- and diaphysis. Independent shape modes were identified using principal component analysis (PCA). Hierarchical cluster analysis of the shape modes was performed to identify natural groupings of patients. Differences in geometric measures of the proximal femur (FO, NSA) and demographic parameters (age, height, weight, BMI) between the clusters were evaluated using Kruskal-Wallis one-way-ANOVA or Chi-square tests, as appropriate.
In the entire cohort, mean FO was 39.0 mm, mean NSA was 131 degrees. PCA identified 10 independent shape modes accounting for over 90% of variation in proximal femoral canal shape within the dataset. Cluster Analysis revealed 6 shape clusters for which all 10 shape modes demonstrated a significantly different distribution (p-range:0.000-0.015). We observed significant differences in age ($p=0.032$), FO ($p<0.001$) and NSA ($p<0.001$) between the clusters. No significant differences with regard to gender or BMI were seen.
Our preliminary analysis has identified 6 different patterns of proximal femoral canal shape which are associated with significant differences in femoral offset, neck-shaft-angle and age at time of surgery. We are currently evaluating the entire dataset of 345 patients which will allow a comprehensive classification of variation in proximal femoral shape and joint geometry. The present data may optimise preoperative planning and improve future implant design in THA.

P102
CHARACTERISATION OF IN SITU CHONDROCYTE DEATH AROUND MECHANICALLY-DRILLED HOLES IN BOVINE ARTICULAR CARTILAGE: IMPLICATIONS FOR INTRA-ARTICULAR INTERNAL FIXATION TECHNIQUES
DA Houston*[1], AK Amin[2], TO White[2], AC Hall[1]
[1]Centre for Integrative Physiology, University of Edinburgh, UK; [2]Department of Orthopaedic and Trauma Surgery, Royal Infirmary of Edinburgh, UK
Intra-articular screw fixation is indicated for internal fixation of large osteochondral fragments secondary to trauma or osteochondritis dissecans. During surgery, orthopaedic drills are used to prepare a hole through which the screw can pass. Previous work has shown that mechanical injury to articular cartilage results in a zone of cell death adjacent to the traumatised articular cartilage (1). Here, we characterise and quantify the margin of in situ chondrocyte death surrounding drill holes and screws (standard cortical and headless compression designs) placed in mature bovine articular cartilage to model the orthopaedic procedure.
Drill holes (1mm) were made through the articular cartilage and bone of intact bovine metacarpophalangeal joints obtained from 3-yr old cows within 12hrs of slaughter. Osteochondral explants (~1cm square and 2-3mm thick) encompassing the drilled holes in articular cartilage and subchondral bone were harvested using a chisel. Explants were then incubated in Dulbecco's modified Eagle's medium for 45mins with CMFDA (5-chloromethylfluorescein diacetate) and PI (propidium iodide; both at 10micromolar) to identify/quantify living and dead in situ chondrocytes respectively in a consecutive series of axial optical sections using confocal scanning laser microscopy (CLSM).
The drill holes through cartilage appeared to have clearly defined edges with no macroscopic evidence of cartilage splitting. However visualisation of fluorescently-labelled in situ chondrocytes by CLSM demonstrated clear cell death around the periphery of the drilled hole which was 166±19 micrometers in width. This increased with a larger diameter (1.5mm) drill to 450±151 micrometers (all data are means±s.e.m.; n=3). Preliminary experiments indicated that the margin of chondrocyte death around a 1.5mm hole was dramatically increased further by the insertion of screws into pre-drilled holes.
These results suggest that the mechanical trauma associated with cartilage drilling and the insertion of intra-articular screws occurs with marked death of in situ chondrocytes extending into normal cartilage beyond the area occupied by the screw. As chondrocytes are not replaced in mature cartilage, their loss around the hole/screw will mean that the extracellular matrix is not maintained, inevitably leading to cartilage failure.
Reference:
(1) Amin, A.K. et al., (2008). J Bone Joint Surg Am 90:1531-1542.

P103
DEVELOPMENT OF A MENISCAL CARTILAGE REPAIR SYSTEM; IN VITRO TESTING UTILISING MENISCAL CARTILAGE CELLS, PLATELET RICH PLASMA AND COLLAGEN BASED SCAFFOLDS
D Howard*[1], J Shepherd[2,3], A Moavenian[3], S Kew[2], S Ghose[2], N Rushton[1], J Wardale[1]

[1]Orthopaedic Research Unit, University of Cambridge, Box 180, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ; [2]Tigenix, Byron House, Cambridge Business Park, Milton Road, Cambridge, CB4 0WZ; [3]Department of Materials Science and Metallurgy, University of Cambridge, New Museum's Site, Pembroke Street, CB2 3QZ, UK

Meniscal cartilage provides joint stabilisation, load distribution, impact absorption and decreased friction in joints that have a complex movement such as the knee. If the meniscal cartilage degrades or is surgically removed, there is a strong probability, over time, of damage to the articular surface. The ability to regenerate damaged meniscal cartilage with an implanted device that replaces the biological equivalent would allow for joint stabilisation, robust movement and reduce the risk of damage to the articular cartilage. An implant with many of the characteristics of meniscus and with the ability to integrate correctly and firmly with the surrounding tissue, would be advantageous.

Inclusion of Platelet Rich Plasma (PRP) into the scaffolds to provide a concentrated source of matrix proteins and autologous growth factors may further enhance the regenerative repair process. To investigate the suitability of the collagen scaffolds, addition of meniscal chondrocytes and or PRP was examined in vitro.

Human meniscal chondrocyte cells were isolated, via collagenase digestion, from meniscal cartilage recovered from total knee replacement surgery. Meniscal chondrocytes were cultured in vitro to expand cell numbers. PRP was produced from volunteer's blood using a centrifuge and density based platelet recovery system. Release of Platelet Derived Growth Factor type AB (PDGF-AB) was measured by ELISA as an indicator of the behaviour of the peptide growth factor component. Combinations of scaffold, meniscal chondrocytes and PRP were tested for interaction, suitability and viability.

Experiments so far have shown good biocompatibility, in vitro, as meniscal chondrocytes were able to grow within the range of scaffolds produced. Cell retention could be enhanced by addition of PRP to the scaffolds. PDGF-AB was released over 5 days from the scaffold and PRP combination.

Further studies are in progress to derive relevant scaffold modifications and combinations for practical, robust, treatment strategies.

P104

LEFT-RIGHT WEIGHT-BEARING: SHORT AND LONG-TERM MEASUREMENT PRECISION, AND EFFECTS OF WEIGHT-BEARING IMBALANCE ON HIP BONE MINERAL DENSITY AND LEG LEAN TISSUE MASS

SJ Hopkins*[1], CW Smith[1], A Toms[2], M Brown[2], JR Welsman[3], KM Knapp[1]

[1]College of Engineering, Mathematics and Physical Sciences, University of Exeter, UK; [2]Princess Elizabeth Orthopaedic Centre, Royal Devon and Exeter Hospital, UK; [3]College of Life and Environmental Sciences, University of Exeter, UK

Weight-bearing is a known stimulus for bone remodelling and a reduction in weight-bearing is associated with reduced bone mineral density (BMD) in affected limbs post lower limb fracture. This study investigated short and long-term precision of a method for measuring relative left/right weight-bearing using two sets of identical calibrated scales. The effect of imbalance on BMD at the hip and on lower limb lean tissue mass (LLTM) was also assessed.

46 postmenopausal women, with no history of leg or ankle fracture, were measured three times whilst standing astride two scales (Seca, Germany). 34 of the participants were re-measured after 6 months by the same method. Bilateral hip and total body dual x-ray absorptiometry measurements were performed using a GE Lunar Prodigy (Bedford, MA). Precision errors in weight-bearing measures were calculated using the root mean square coefficient of variation (RMSCV%).The correlations at the first visit between left/right differences in weight-bearing and differences in BMD and LLTM were calculated.

The short-term RMSCV% for left and right weights were 4.20% and 4.25% respectively and the long-term RMSCV% were 6.91% and 6.90%. Differences in left/right weight-bearing ranged from 0 to 24% (SD 8.63%) at visit 1 and 0 to 30% (SD 10.71%) at visit 2. Using data from visit 1, the relationship between hip BMD differences and left/right weight-bearing differences were investigated, with no significant correlations found. However, a weak, but statistically significant correlation of r=0.35 (p=0.02) was found for differences in LLTM and left/right weight-bearing differences.

In conclusion, left/right weight-bearing measured using two scales is a precise method for evaluating differences in weight-bearing in the short and long-term. Differences in left/right weight-bearing in this population varied by up to 30%. Participants showed a high degree of consistency in their long-term balance in a natural standing posture. Inequalities in left/right weight-bearing did not correlate significantly with BMD at the hip, but demonstrated a weak but statistically significant correlation with lean tissue mass.

P105

PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY TO STUDY SKELETAL MATURITY

KA Ward*[1], J Thompson[1], D Harnpanich[1], M Ceesay[2], M Mendy[2], A Prentice[1,2]

[1]MRC Human Nutrition Research, Cambridge, UK; [2]MRC Keneba, The Gambia

Growth plate fusion is a marker of cessation of longitudinal growth. The appearance and disappearance of growth plates is used to give a child a bone age which reflects their growth relative to maturity rather than chronological age. In Africa stunting is common and growth velocities often slower. Qualitative observations have suggested growth occurs well into the third decade. The present study aimed to i) determine whether peripheral quantitative computed tomography (pQCT) could be used to study growth status and ii) whether there is evidence for growth in females who have had at least one pregnancy).

As part of an on-going study of skeletal phenotype in The Gambia we have acquired a longitudinal pQCT scans at the radius and tibia. A scout view is taken of the distal site to determine scan location; from this is it possible to visualise the growth plate. A scoring system was developed grading scout views as open, partially fused, or fused.

Males and females aged 17-25 years old were scanned; 3 groups were studied males (M), females who were non pregnant, none lactating (NPNL), females who had at least on pregnancy (PF). At time of last measurement 77% M, 100% NPNL had fused growth plates at radius, 19% M were partially fused. At the tibia 30%, 0% fused; 70% and 100% partially fused in M & NPNL respectively. Similar patterns were found in PF whereby tibia was less mature than the radius and even those who had at least on reproductive cycle there was evidence for partially fused growth plate. During the study period, statural height increased in the study population, particularly in boys.

These data demonstrate evidence for continued growth in young Gambian adults with a difference in the timing of completion of growth between non weight bearing and weight bearing sites. There also appeared to be evidence of growth in females with at least one reproductive cycle. The consequences of this for nutrient partitioning between mother and child requires follow-up. The implications of these observations for development of a healthy skeletal phenotype and timing of interventions require further study.

P106

EXPERIMENTAL VALIDATION OF A FREE BOUNDARY CONDITION FINITE ELEMENT MODEL OF THE FEMUR

AM Younge*[1], ATM Phillips[1], AA Amis[2]

[1]Department of Civil and Environmental Engineering, Imperial College London, UK; [2]Department of Mechanical Engineering, Imperial College London, UK

Finite element models of the musculoskeletal system have the possibility of describing the in vivo situation to a greater extent than a single in vitro experimental study ever could. However these models and the assumptions made must be validated before they can be considered truly useful. The object of this study was to validate, using digital image correlation (DIC) and strain gauging, a novel free boundary condition finite element model of the femur.

The femur was treated as a complete musculoskeletal construct without specific fixed restraint acting on the bone. Spring elements with defined force-displacement relationships were used to characterize all muscles and ligaments crossing the hip and knee joints. This model was subjected to a loading condition representing single leg stance. From the developed model muscle, ligament and joint reaction forces were extracted as well as displacement and strain plots. The muscles with the most influence were selected to be represented in the simplified experimental setup.

To validate the finite element model a balanced in vitro experimental set up was designed. The femur was loaded proximally through a construct representative of the pelvis and balanced distally on a construct representing the tibio-femoral joint. Muscles were represented using a cabling system with glued attachments. Strains were recorded using DIC and strain gauging. DIC is an image analysis technique that enables non-contact measurement of strains across surfaces. The resulting strain distributions were compared to the finite element model.

The finite element model produced hip and knee joint reaction forces comparable to in vivo data from instrumented implants. The experimental models produced strain data from both DIC and strain gauging; these were in good agreement with the finite element models. The DIC process was also shown to be a viable method for measuring strain on the surface of the specimen.

In conclusion a novel approach to finite element modeling of the femur was validated, allowing greater confidence for the model to be further developed and used in clinical settings.

P107

STRUCTURAL ANALYSIS OF THE FEMUR A COMPLIMENTARY APPROACH TO THE FINITE ELEMENT METHOD

AM Younge*[1], ATM Phillips[1], AA Amis[2]

[1]Department of Civil and Environmental Engineering, Imperial College London, UK; [2]Department of Mechanical Engineering, Imperial College London, UK

Finite element (FE) modelling has been widely used to create and assess musculoskeletal models. However to achieve a high degree of resolution in describing the structure, significant computational power and time are required. The objective of this study was to introduce a complimentary approach to FE

modelling using structural beam theory. This requires far less computational power and models can be analyzed in a fraction of a second, offering quick, intuitive results for engineers and surgeons.
Beam theory was first introduced as a method for analyzing the stresses in long bones in 1917. It was used as the de facto method for several decades. The introduction of FE modelling offered great advances; beam theory calculations were considered laborious and less accurate. However with the advances in computational power so too comes the ability to create modern automated beam theory models.
A study was conducted using the commercially available general structural analysis software Oasys GSA. A synthetic biomechanical femur was CT scanned and the solid model constructed. This model was sectioned into approximately seventy sections in the regions of the shaft and condyles, thirty in the neck and thirty in the head. Line plots of the shape of each of the sections, for both cortical and trabecular parts, were then imported into Oasys GSA. The centroid, area, second moments of area and torsion constant were calculated for each section. The sections were plotted at the position of the cortical centroid and parallel axis theorem was used to plot the trabecular section in the same position. A force representing the hip joint reaction force was applied to a node corresponding to the centre of the femoral head. Muscular forces were applied to stiff radial elements according to those active at the point of peak joint contact force during gait.
Oasys GSA produced instant results showing moment and deflection characteristics of the femur. This data was then used to predict strain plots, which were directly compared to FE results. Initial results compare favourably.
This study has demonstrated an updated fast, efficient and intuitive alternative to finite element modelling.

P108
THE IMPORTANCE OF MODELLING BONE-IMPLANT INTERFACE IN LOCKING PLATE FE MODELS
A MacLeod*, P Pankaj, H Simpson
Department of Orthopaedic Engineering, University of Edinburgh, UK
Finite element modelling is being extensively used to evaluate the biomechanical behaviour of fractured bone treated with fixation devices. Appropriate modelling of the bone-implant interface is key to quality biomechanical prediction.
The present study considers this interface modelling in the context of locking plates. A majority of previous studies assume the interface to be represented by a tied constraint or a fully bonded interface. Many other studies incorporate a frictional interface but ignore screw threads. This study compares the various interface modelling strategies. An interface with screw threads explicitly included is also considered.
The study finds that interface modelling has significant impact on both the global and local behaviour. Globally, the load-deflection behaviour shows considerable difference depending on the interface model. Locally, the stress-strain environment within the bone close to the screws is significantly altered.
The results show that the widely used tie constraint can overestimate stiffness of a construct which must be correctly predicted to avoid non-union or periprosthetic re-fracture, especially in osteoporotic bone. In addition, the predictions of screw loosening, bone damage and stress shielding are very different when screw threads are included in the model.

P109
FOLLOW-UP OF GAMBIAN RICKETS: POSSIBLE AETIOLOGICAL FACTORS
V Braithwaite*[1], LMA Jarjou[2], GR Goldberg[1,2], S Nigdikar[1], H Jones[1], A Prentice[1,2]
[1]MRC Human Nutrition Research, Cambridge, UK; [2]MRC Keneba, The Gambia
A case-series of children (n=46) with suspected calcium-deficiency rickets presented at MRC clinics in The Gambia with rickets-like bone deformities. Biochemical analyses discounted vitamin D-deficiency as an aetiological factor but indicated a perturbation of Ca-P metabolism involving low plasma phosphate and high circulating C-terminal fibroblast growth factor-23 (FGF23) [1]. The children were treated with calcium and vitamin D for 6-12 months. A follow-up study was conducted 4-5 years after presentation to investigate possible aetiology and characterise recovery.
35 children (RFU) consented to follow-up. Standardised clinical examinations to assess severity of bone deformities, 2hr fasting urine and blood sample collection, 48hr weighed dietary analysis and 24hr urine collections were made. Data from children from the local community (LC) were used to calculate standard deviation scores (SDS) to control for age, sex and season.
None of RFU had radiological signs of active rickets. However, 54% (n=19) had visible leg deformities consistent with rickets, including 4 whose deformity had changed from genu varum to valgum, while the others had no lasting deformity.
Differences in biochemical and dietary profile were observed between RFU and LC. These included lower dietary calcium intake (SDS=-0.5, p=0.02), daily urinary calcium excretion (SDS=-0.7, p=0.03), TmP:GFR (SDS=-0.5, p=0.04) and eGFR-cystatinC (SDS=-0.9, p=0.03). FGF23 tended to be higher (SDS=0.4, p=0.5). Haemoglobin (Hb) was a negative predictor of FGF23 in RFU (lnFGF23=21.9-7.14lnHb, R2=26.6%, p=0.006) but not in LC (lnFGF23=0.43+1.47lnHb, R2=4.8%,p=0.3). There was an Hb x group interaction (p=0.003) demonstrating a difference in slope in the relationship between Hb and FGF23 in the two groups.
Differences were apparent between RFU with and without lasting leg deformities. These included higher circulating 1,25OH2D (SDS=0.6, p=0.05) and magnesium (SDS=0.3, p=0.008) and lower eGFR-cystatinC (SDS=-0.9, p=0.01) in those with lasting deformity.
In conclusion, differences in biochemical and dietary profile exist between Gambian children 4-years after initial presentation with rickets and children from the local community. In addition, biochemical differences are apparent in those who recover from bone deformities and those who do not. Our results suggest low dietary calcium intake, poor glomerular filtration and low iron status as potential aetiological factors in Gambian Rickets.
1. Prentice. Bone2008.42:p.788-797.

P110
BONE REMODELLING AND APOSITION ONTO CALCIUM PHOSPHATE COATED POLYMER SURFACES IN THE SPINE: AN IN VIVO ANIMAL MODEL
T Steffen*[1], BJC Freeman[2], M Aebi[3]
[1]McGill University, Canada, [2]University of Adelaide, Australia, [3]University of Bern, Switzerland
Long term, secondary implant fixation of Total Disc Replacements (TDR) can be enhanced by hydroxyapatite or similar osseo-conductive coatings. These coatings are routinely applied to metal substrates. The objective of this in vivo study was to investigate the early stability and subsequent bone response adjacent to an all polymer TDR implant over a period of six months in an animal model.
Six skeletally mature male baboons (Papio annubis) were followed for a period of 6 months. Using a transperitoneal exposure, a custom-sized Cadisc L device was implanted into the disc space one level above the lumbo-sacral junction in all subjects. Radiographs of the lumbar spine were acquired prior to surgery, and post-operatively at intervals up to 6 months to assess implant stability. Flourochrome markers (which contain molecules that bind to mineralization fronts) were injected at specified intervals in order to investigate bone remodeling with time.
Animals were humanely euthanized six months after index surgery. Test and control specimens were retrieved, fixed and subjected to histological processing to assess the bone-implant-bone interface. Fluorescence microscopy and confocal scanning laser microscopy were utilized with BioQuant image analysis to determine the bone mineral apposition rates and gross morphology.
Radiographic evaluation revealed no loss of disc height at the operative level or adjacent levels. No evidence of subsidence or significant migration of the implant up to 6 months. Heterotopic ossification was observed to varying degrees at the operated level.
Histology revealed the implant primary fixation features embedded within the adjacent vertebral endplates. Flourochrome distribution revealed active bone remodeling occurring adjacent to the polymeric end-plate with no evidence of adverse biological responses. Mineral apposition rates of between 0.7 and 1.7 microns / day are in keeping with literature values for hydroxyapatite coated implants in cancellous sites of various species.
Radiographic assessment demonstrates that the Cadisc L implant remains stable in vivo with no evidence of subsidence or significant migration. Histological analysis suggests the primary fixation features are engaged, and in close apposition with the adjacent vertebral bone. Flourochrome markers provide evidence of a positive bone remodelling response in the presence of the implant.

P111
DEVELOPMENT OF AN ORTHOTROPIC BIOFIDELIC MODEL OF THE WHOLE FEMUR
DM Geraldes*, ATM Phillips
Structural Biomechanics Group, Dept. Civil and Environmental Engineering, Imperial College London, London, UK
Recently finite element studies have incorporated bone remodelling algorithms in an attempt to simulate bone's mechano-adaptation to loading conditions. In order to simplify these analyses, bone is usually considered to be isotropic, which does not explain the directionality of its internal structures; neither the orthotropic properties measured at the continuum level. Furthermore, simplified loading is usually applied to the bone models, which can result in an unrealistic remodelling stimulus. However, free boundary condition modelling of the femoral and pelvic constructs has been shown to produce more physiological stress and strain distributions.
This paper describes the application of a 3D remodelling algorithm (with bone modelled as a strain-adaptive continuum with local orthotropic material properties) to a free boundary model of the femoral construct, where the hip and knee joints, as well as muscles and ligaments crossing the joints were included explicitly. Two load cases were analysed: single leg stance and standing up.
Material properties and directionality distributions were produced for the whole femur, showing good agreement with observed structures from clinical studies. This indicates that the loading conditions modelled correspond to those experienced in vivo. In addition, the impact of the different load cases in bone

structure modelling could be compared. Observations of the material properties distribution and orientation for standing up indicate that it promotes changes in bone stiffness in the anterior regions of the femoral neck and cortical shaft and the posterior side of the condyles.
Development of this approach to modelling and bone structure prediction can lead to a better understanding of bone's mechanical behaviour and to the development and public release of orthotropic heterogeneous models for different constructs. These can be applied in many areas of interest in orthopaedic biomechanics, such as the study of bone-implant interfaces, improvement of the currently used surgical tools and techniques and the influence of certain activities in affecting local bone strength and mineralisation.

P112
HUMAN ARTICULAR CARTILAGE-DERIVED PROGENITOR CELLS: IDENTIFICATION AND CHARACTERISATION
R Williams*[1], IM Khan[1], K Richardson[1], L Nelson[1], D Baird[2], S Roberts[3], J Dudia[4], T Briggs[5], J Fairclough[6], CW Archer[1]
[1]Pathophysiology and Repair Dept, Cardiff University, UK; [2]Pathology Dept, Cardiff University, UK; [3]Cytogenetics Dept, University Hospital of Wales, UK; [4]Dept of Veterinary Clinical Sciences, Royal Vet College, UK; [5]Dept of Orthopaedics, Llandough Hospital, Uk; [6]Royal Orthopaedic Hospital, Stanmore, UK
Hyaline cartilage defects are a significant clinical problem for which a plethora of cartilage repair techniques are used. One such technique is cartilage replacement therapy using autologous chondrocyte or mesenchymal stem cell (MSC) implantation (ACI). Mesenchymal stem cells are increasingly being used for these types of repair technique because they are relatively easy to obtain and can be expanded to generate millions of cells. However, implanted MSCs can terminally differentiate and produce osteogenic tissue which is highly undesirable, also, MSCs generally only produce fibrocartilage which does not make biomechanically resilient repair tissue, an attribute that is crucial in high weight-bearing areas. Tissue-specific adult stem cells would be ideal candidates to fill the void, and as we have shown previously in animal model systems [Dowthwaite et al, 2004, J Cell Sci 117;889], they can be expanded to generate hundreds of millions of cells, produce hyaline cartilage and they have a restricted differential potential. Articular chondroprogenitors do not readily terminally differentiate down the osteogenic lineage.
At present, research focused on isolating tissue-specific stem cells from articular cartilage has met with modest success. Our results demonstrate that using differential adhesion it is possible to easily isolate articular cartilage progenitor populations from human hyaline cartilage and that these cells can be subsequently expanded in vitro to a high population doubling whilst maintaining a normal karyotype. Articular cartilage progenitors maintain telomerase activity and telomere length that are a characteristic of progenitor/stem cells and differentiate to produce hyaline cartilage.
In conclusion, we propose the identification and characterisation of a novel articular cartilage progenitor population, resident in human cartilage, which will greatly benefit future cell-based cartilage repair therapies.

P113
LIVE CELL IMAGING AND MATHEMATICAL MODELLING REVEALS A ROLE FOR NON-CANONICAL TGFBETA SIGNALLING IN MATRIX PROTEIN TRAFFICKING
D Ribe*[1], A Tucker[2], G Stenbeck[1]
[1]Centre for Cell and Chromosome Biology, Brunel University, UK; [2]School of Information Systems, Computing and Mathematics, Brunel University, UK
Intracellular trafficking of matrix proteins prior to secretion is vital for bone remodelling and under the control of cytokines and growth hormones. However, little is known how these extracellular signals modulate the intracellular trafficking machinery. In disease states such as osteoarthritis, which are also characterised by aberrant matrix deposition, osteoblasts show reduced responsiveness to transforming growth factor beta (TGFbeta). TGFbeta exerts its effects through diverse signalling pathways, including the Smad, Rho, PI-3 kinase and MAP kinase pathways. Our study aims to identify the immediate early effects of TGFbeta on intracellular trafficking of bone matrix proteins and the molecular machinery responsible using total internal reflection microscopy (TIRFM) and statistical computer modelling.
ROS17/2.8 cells were transfected with a plasmid for osteonectin(ON)-GFP expression and ON-containing vesicles were visualised by TIRFM. Serial TIRFM images were collected and analysed using the Particle Tracker plugin for ImageJ. Mean square displacements (MSD) were calculated and used to classify vesicle trajectories as directed diffusion, simple diffusion and restricted diffusion.
Analysis of cells treated with either vehicle, 5 µM of the MEK inhibitor UO126, 10 µM of the p38 MAPK inhibitor BIRB796, 100 nM of the PI3-kinase inhibitor wortmannin or 10 µM of the ROCK inhibitor Y-27632 for 30 min before subsequent TGFbeta treatment for 10 min showed that TGFbeta caused a 3.4 ± 0.7 fold increase in slope of MSD curves of ON-GFP vesicle trajectories, which was inhibited in the presence of U0126, BIRB796, Y-27632 but not by wortmannin. Analysis with Auto Regressive Hidden Markov models on the control and TGFbeta treatment data showed that one hidden state is dominant and that TGFbeta treatment data generates models that are less stable with a high probability of state changes. A decision tree classifier was constructed and resulted in a fit to the experimental data with 96% accuracy.
Our results show that non-classical TGFbeta signalling has an immediate effect on trafficking of the secreted protein ON, where ROCK, MEK and p38 MAP kinase signalling regulate vesicle trajectories and that our probabilistic models are powerful tools to analyse the complex intracellular movement of vesicules carrying matrix proteins.

P114
LOADING OF THE HUMAN KNEE ALTERS SYNOVIAL FLUID METABOLITE PROFILES
Roberts HC[1]*, Chowdhury R[1] Paisey S[1], Wilson C[2], Mason DJ[1]
[1]School of Biosciences, Cardiff University, Museum Avenue, Cardiff; [2]UHW, Heath Park, Cardiff, UK
Purpose of study: To determine whether cycles of pivot shift testing prior to anterior cruciate ligament (ACL) reconstruction alters metabolite levels in synovial fluid.
Method: Testing for pivot shift is a standard aspect of the EUA prior to an ACL reconstruction. Teaching 2 trainees to perform the pivot test will result in the knee being pivoted 5 times. All cases were isolated ACL deficiency, without meniscal or chondral damage (n=3). Each knee had synovial fluid extracted under aseptic conditions following anaesthesia. The pivot shift test was then performed and demonstrated 5 times. After preparation of the knee for surgery, a second synovial fluid sample was extracted. The time between samples was 5 minutes. Synovial fluids were analysed using 500 MHz 1H NMR spectroscopy. Chemical shifts were referenced to known concentration NMR internal standard (TSP), peaks identified and peak integrals measured using the Bruker software Topspin 2.0.
Results: NMR revealed 26 metabolite-specific peaks in synovial fluid spectra. Some specific metabolite concentrations varied in response to pivot shift testing. For example, we found increases of up to 94% lactate, 48% n-acetyl glycoproteins, 14% arginine, 44% alanine, 38% CH lipids and 45% valine levels in synovial fluid following pivot shifting.
Conclusion: Our pilot data indicates that the metabolic profile of synovial fluid varies before and after pivot shift testing. The results suggest that low energy shear force in the ACL deficient knee, in the absence of meniscal or chondral damage, is sufficient to alter metabolite levels in the synovial fluid. This may represent the first indication of specific metabolites that change in response to altered biomechanical loading in the human knee.

P115
INTERNAL DISC DISRUPTION: THE ROLE OF STRESS GRADIENTS
M Stefanakis[1], J Luo[2], P Pollintine [1], P Dolan[1], MA Adams[1]*
[1] Centre for Comparative and Clinical Anatomy, University of Bristol, UK; [2] Roehampton University, UK
Background: In the annulus fibrosus of degenerated intervertebral discs, disruption to inter-lamellar cross-ties appears to lead to delamination, and the development of anulus fissures. We hypothesise that such internal disruption is likely to be driven by high gradients of compressive stress (i.e. large differences in stress from the nucleus to the mid anulus).
Methods: Eighty-nine thoracolumbar motion segements, from T7/8 to L4/5, were dissected from 38 cadavers aged 42-96 yrs. Each was subjected to 1 kN compressive loading, while intradiscal compressive stresses were measured by pulling a pressure transducer along the disc's mid-sagittal diameter. Measurements were repeated in flexed and extended postures. Stress gradients were measured, in the anterior and posterior anulus of each disc, as the average rate of increase in stress (MPa/mm) between the nucleus and the region of maximum compressive stress in the anulus. Average nucleus pressure (IDP) was also recorded.
Results: Stress gradients increased with grade of disc degeneration, especially in the posterior anulus ($p<0.04$ or better). Age had little additional influence, despite an inverse correlation with IDP ($p<0.04$). Stress gradients increased in the anterior anulus in flexion, and were greatest of all in the posterior anulus in extension, sometimes exceeding 0.5 MPa/mm. In the most severely degenerated discs, stress gradients remained high, even though peak anulus stresses and IDP were reduced as a result of load-bearing being transferred to the neural arch.
Discussion: Stress gradients are highest in the region of the disc (the middle posterior anulus) that is most disrupted by the degenerative process. Unlike the overall peak stresses, or IDP, stress gradients remain high in severely degenerated discs, and are not reduced when load-bearing is transferred to the neural arch. These results suggest that stress gradients play a major role in the internal disruption of degenerated human discs.
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P116
CREEP' DEFORMITY OF FRACTURED VERTEBRAE IS REDUCED BY VERTEBROPLASTY
J Luo*[1], DJ Annesley-Williams[2], MA Adams[1], P Dolan[1]
[1]Centre for Comparative and Clinical Anatomy, University of Bristol, Bristol, UK; [2]Queen's Medical Centre, Nottingham, UK

Vertebral osteoporotic fracture increases both elastic and time-dependent ('creep') deformations of the fractured vertebral body during subsequent loading. The accelerated rate of creep deformation is especially marked in central and anterior regions of the vertebral body where bone mineral density is lowest. In life, subsequent loading of damaged vertebrae may cause anterior wedging of the vertebral body which could contribute to the development of kyphotic deformity. The aim of this study was to determine whether gradual creep deformations of damaged vertebrae can be reduced by vertebroplasty.
Fourteen pairs of spine specimens, each comprising three vertebrae and the intervening soft tissue, were obtained from cadavers aged 67-92 yr. Specimens were loaded in combined bending and compression until one of the vertebral bodies was damaged. Damaged vertebrae were then augmented so that one of each pair underwent vertebroplasty with polymethylmethacrylate cement, the other with a resin (Cortoss). A 1kN compressive force was applied for 1 hr before fracture, after fracture, and after vertebroplasty, while creep deformation was measured in anterior, middle and posterior regions of each vertebral body, using a MacReflex optical tracking system.
Cement type had little influence on creep deformation, so data from all 28 specimens were pooled. After fracture, creep in the anterior vertebral body increased from 4,513 (STD 4766) to 54,107 (STD 54,845) microstrains ($P<0.001$), and creep in the central region of the vertebral body increased from 885 (STD 5,169) to 34,378 (STD 40,762) microstrain ($P<0.001$). (10,000 microstrains = 1% deformation.) Following vertebroplasty, creep deformations were reduced by 61% ($P=0.002$) and 66% ($P=0.006$) in anterior and central regions respectively.
Creep deformations of the anterior and central regions of vertebral bodies increase markedly as a result of fracture but are then reduced by vertebroplasty. In life, vertebroplasty could help to slow or prevent the gradual development of kyphotic deformity following vertebral osteoporotic fracture, as well as increase vertebral stiffness and strength.

P117
EARLY FAILURE OF CERAMIC-ON-METAL HIP PROSTHESES IN VIVO
TJ Joyce*[1], J Lord[1], DJ Langton[1,2], AVF Nargol[2]
[1]Newcastle University, UK; [2]University Hospital of North Tees, UK
Introduction
Total hip prostheses which use a ceramic head within a metal liner are a relatively recent introduction. As such, survivorship rates from independent centres alongside explant analysis are rare. The early experience with this novel ceramic-on-metal (CoM) bearing couple is reported.
Methods and Materials
All CoM hips implanted between 2008 and 2009 at a single hospital by a single surgeon were reviewed. Radiographs were analysed using EBRA software to determine acetabular cup inclination and anteversion angles. Blood metal ion concentrations were measured using inductively coupled plasma mass spectroscopy (ICPMS). Explants were measured for bearing surface and taper wear using a high precision co-ordinate measuring machine. The roughness of the articulating surfaces was measured with a non-contact profilometer.
Results
In 54 patients 56 CoM hips were implanted. Mean (range) age was 64 years (34-87). There were 41 females and 15 males. Patients were followed-up for a mean of 1.5 years. Three hips were revised at mean of 1.2 years (2 female, 1 male) with a further 3 listed for revision under 1.5 years giving an overall failure rate of 10.7%. All these patients reported with pain. X-rays of failed devices showed a characteristic pattern of femoral stem loosening. Serum cobalt and chromium were less than 2 micrograms/L. Explant analysis of the three revised hips showed wear at the liner rim in each case. In two of these cases the wear extended completely around the circumference. The wear volumes were 4.1, 2.0 and 2.3mm3 respectively. The ceramic heads were unworn but some transfer of metal could be seen visually. There was no significant wear or deformation at the taper junctions. Typical ceramic head roughness values were 3nm Ra and so most of the surface area of the heads remained in a pristine condition.
Discussion
The very high early failure rate using COM is concerning. Explant analysis suggests equatorial contacts with propagation of high frictional forces distally. These forces may have caused early loosening of the femoral stems. Orthopaedic surgeons need to be aware of this new mechanism of failure which is associated with low metal ions.

P118
BONE CELL NETWORK VIABILITY: NOVEL 3D MAPPING OF CANCELLOUS OSTEOCYTE INTERCONNECTION IN THE AGEING HUMAN FEMORAL HEAD
PE Garner*[1,2], RK Wilcox[2], JE Aaron[1]
[1]IMSB, FBS, University of Leeds, UK; [2]IMBE, University of Leeds, UK
Osteocytes are the most abundant bone cell (90-95%) and in normal tissue each may remain viable for many years. Despite entombment in calcified matrix their extensive processes form a pervasive syncytium, similar to that of neurones in the central nervous system, and whereby no part of bone is more than a few microns from an osteocyte. This suggests that they are not simply passive, but that they have a role in mechanotransduction, influencing remodelling and self-repair and functioning as the skeletal mechanostat (Frost 2003). To gain insight into the potential morphological variability of the network with reference to skeletal location and to associated stress input, a novel method has been developed combining undecalcified histology, confocal microscopy and image analysis software that enables the quantitative characterisation of the major 3D network features. The method has been subsequently applied to quartered, ageing human femoral heads (a particularly vulnerable skeletal site) to identify any regional differences (e.g. relative to tendon insertion proximity versus compression sites) and also to compare the network in contrasting conditions of traditionally high stress (osteoarthritis) and low stress (osteoporosis). Bone segments underwent en-bloc staining in the fluorochrome 0.1% calcein for 3 days under vacuum before embedding in methylmethacrylate resin. Thick (300 microns) undecalcified slices were cut and scanned and image z-stacks (approx. 200-250 x-y images 0.3 microns apart) captured using a laser scanning confocal microscope with a krypton/argon laser, a 500nm excitation filter and 700nm emission filter. Individual x-y 2D Tiff images were imported into Simpleware (analytical software) to generate a complementary pair of 3D binary masks representative of either the osteocyte cell bodies alone or of the extensive processes alone. Corresponding in-house code (Matlab, Mathworks, USA) was written to quantitate the complementary paired masked aspects including the volume of the cell body component, the number of processes/cell, their length, interconnection and anisotropy. The objective outcome will augment previous subjective appraisal of the syncitium, leading to a more precise understanding of its capacity for musculoskeletal exchange and bone mass determination in ageing and disease.

P119
HIGH FAILURE RATES OF THE ASR HIP PROSTHESIS
TJ Joyce*[1], DJ Langton[1,2], J Lord[1], AVF Nargol[2]
[1]Newcastle University, UK; [2]University Hospital of North Tees, UK
The worldwide withdrawal of the DePuy Articular Surface Replacement (ASR) device in both its resurfacing and total hip replacement (THR) form on 26 August 2010, after 93,000 were implanted worldwide, has had major implications. The 2010 National Joint Registry for England and Wales quoted figures of 12-13% failure at five years; however these figures may be an underestimate.
In 2004 a single surgeon prospective study of the ASR bearing surface was undertaken. Presented are the Adverse Reaction to Metal Debris (ARMD) failure rates of the ASR resurfacing and ASR THR systems. The diagnosis of ARMD was made by the senior author and was based on clinical history, examination, ultrasound findings, metal ion analysis of blood and joint fluid, operative findings and histopathological analysis of tissues retrieved at revision. Acetabular cup position in vivo was determined using EBRA software. Mean follow up was 52 months (24-81) and 70 patients were beyond 6 years of the procedure at the time of writing. Kaplan Meier survival analysis was carried out firstly with joints designated 'failure' if the patient had undergone revision surgery or if the patient had been listed for revision. A second survival analysis was carried out with a failure defined as a serum cobalt concentration > 7microgrammes/L (MHRA guideline from MDA-2010-069). Full explant analysis was carried out for retrieved prostheses.
There were 505 ASR hips in total (418 resurfacings and 87 THRs). 657 metal ion samples were available at the time of writing including 152 repeats. Survival analysis using revision/listed for revision as end point (at 6 years): ASR resurfacing: 26.1% failure; ASR THR: 55.5% failure. Survival using ion analysis (at 5 years): ASR resurfacing: 50.1% failure; ASR THR: 66.5% failure. The median (range) volumetric wear rate of failed prostheses was 8.23mm3/year (0.51-95.5). Failure and high ion concentrations are linked to acetabular cup size, anteversion and inclination. Increased failure rates in THRs were due to wear at the taper junction of head and stem.
Design flaws in the ASR have led to excessive wear and consequently catastrophic failure rates secondary to ARMD.

P120
A DIRECT METHOD FOR THE FOR THE SPATIAL 3D MAPPING OF TRABECULAR TERMINI IN THE SPINE
PE Garner*[1,2], RK Wilcox[2], JE Aaron[1]
[1]IMSB, FBS, University of Leeds, UK; [2]IMBE, University of Leeds, UK
Bone loss with age is insidious, leaving the skeleton fracture-prone in an ever growing elderly population. Traditionally the bone mineral density (BMD) has been used to predict atraumatic fracture risk as a low bone mass was considered the major predisposing factor. However with frequent reports of overerlap in BMD between fracture and non-fracture groups, the relationship between bone quantity and bone quality (bone strength within the microarchitecture) may provide a more reliable risk appraisal. The development of an accurate method of cancellous connectivity assessment, (as a significant factor in structural strength) in 2D and 3D is challenging; currently none is ideal. Traditional histological methods to provide topographical data from the microarchitecture are limited as they only indirectly assess 3D structural quality due to their use of thin (8 microns) undecalcified sections. Aaron et al (2000) developed a thick (300 microns) slicing and superficial staining method whereby real trabecular termini (ReTm) are identified directly in their 3D context. To extrapolate from this previous method, computer algorithms and MicroCT scanning are now combined to aid in the spatial 3D mapping of ReTm as loci of structural

weakness independent of the bone mass. Embalmed vertebral bodies were MicroCTCT scanned, hemi-sected, plastic embedded, thickly (300 microns) sliced, superficially stained with silver nitrate and the ReTm mapped using light microscopy and assigned coordinates which corresponded to 9 predetermined topographical regions. A one-way test of variance (ANOVA) showed their distribution to be heterogeneous. These ReTm were then visualised in 3D using code developed in-house (Matlab, Mathworks, USA) in the form of a transparent 3D shell corresponding to the cortex, with ReTm enclosed within. To refine and validate this cortical shell, the TIFF images generated from the initial MicroCT scan were used to generate another 3D cortical shell with a surface topography and the coordinate data overlaid; little disagreement in shape was found. This novel method offers the capacity to visualise ReTm spatial within a 3D framework surmounting the constraints of established 2D histology. ReTm distribution within the spine is now being mapped to provide further insight into apparently intransigent atrophy as a major cause of disability.

P121

MULTI-FACTORIAL FE ANALYSIS OF METAL ON METAL ACETABULAR CUP FAILURE

SG Clarke*[1], ATM Phillips[1]
[1]Department of Civil and Environmental Engineering, Imperial College London, UK

Metal on metal press-fit acetabular cups are the worst performing acetabular cup type with severe failure consequences compared to cups made from more inert materials such as polyethylene or ceramic. The cause of failure of these cup types is widely acknowledged to be multi-factorial, therefore creating a complex scenario for analysis through clinical studies. A factorial analysis has been carried out using an experimentally validated finite element analysis to investigate the relative influence of four input factors associated with acetabular cup implantation on output parameters indicating potential failure of the implantation. These input factors were: cup material stiffness; cup inclination; cup version; cup seating; and level of press-fit. The output parameter failure indicators were: wear; tensile strains in the underlying bone; bone remodelling; and cup-bone micromotions.

The factorial analysis concluded that the most significant influence was that of cup inclination on wear, and the second most significant was the influence of the level of press-fit on bone remodelling at the acetabular rim. Significant influence was also observed between version angle and wear, and cup-seating and micro-motion.

The results demonstrated the clear multi-factorial nature of implant failure and highlighted the importance of correct implant positioning and fit.

P122

HUMAN DERIVED OSTEOARTHRITIC CARTILAGE PROGENITOR CELLS ELICIT IN VITRO REGENERATIVE CAPACITY

L Nelson*[1], HE McCarthy[1], J Fairclough[1], CW Archer[1]
Cardiff University, School of Biosciences, Museum Avenue, Cardiff, CF10 3AX, UK

Osteoarthritis (OA) is the most common form of joint disease leading to disability and dependence. In severe cases of knee OA, the joint is deemed irrecoverable and total knee replacements are indicated. Tissue engineering is a possible solution for this pathology and previous work from our laboratory has demonstrated that it is possible to isolate and expand chondroprogenitor cells in vitro from healthy knee-joint articular cartilage. Work presented here describes the detection and isolation of chondroprogenitor cells derived from osteoarthritic cartilage following total knee replacement in patients with severe OA, suggesting a pool of viable cells from this degenerate region which has been previously deemed non-recoverable.

Human articular cartilage was excised from tibial plateaux (TP's) obtained from total knee replacements following the diagnoses of severe OA. Cells were isolated by a sequential pronase and collagenase digestion and subject to a fibronectin adhesion assay. Cells were expanded in monolayer in supplemented growth medium. Clonal 3D pellet cultures were established in chondrogenic and osteogenic differentiation media. Adipogenic cultures were also established in monolayer cultures. Histological procedures, immunohistochemistry and molecular biology were undertaken in order to determine the extent of differentiation. In addition, osteochondral plugs were excised from the TP's and wax embedded for further histological and immunohistochemical analysis.

Clonal cell lines obtained from osteoarthritic knee-joint cartilage using the fibronectin adhesion assay were isolated and successfully cultured to a maximum of 60 population doublings whilst still demonstrating a chondrogenic capacity. Three-D pellet cultures after 21 days of chondrogenic induction produced smooth and iridescent pellets which stained positively for toluidine blue and safranin O. Positive labelling for collagen type II and aggrecan were also observed. Following osteogenic induction; evidence of mineralisation was indicated by the von Kossa stain. Adipogenic induction revealed a positive result. Osteochondral plugs demonstrated sporadic positive labelling in the surface region for putative stem cell marker Stro-1.

Chondroprogenitor cells isolated from osteoarthritic display a strong chondrogenic phenotype, and have the ability to be induced into different lineages. These findings suggest the presence of a pool of viable chondroprogenitors from osteoarthritic tissue which was otherwise deemed irrecoverable.

P123

INGROWTH OF NERVES AND BLOOD VESSELS INTO PAINFUL INTERVERTEBRAL DISCS

M Stefanakis*, I Sychev, BA Summers, P Dolan, I Harding, MA Adams
Centre for Comparative and Clinical Anatomy, University of Bristol, Bristol UK

Introduction: Severe 'discogenic' back pain may be related to the ingrowth of nerves and blood vessels, although this is controversial. We hypothesise that ingrowth is greater in painful discs, and is facilitated in the region of annulus fissures.

Methods: We compared tissue removed at surgery from 22 patients with discogenic back pain and/or sciatica, and from 16 young patients with scoliosis who served as controls. Wax-embedded specimens were sectioned at 7µm. Nerves and blood vessels were identified using histological stains, and antibodies to PGP 9.5 and CD31 respectively.

Results: Blood vessels were identified in 77% of 'painful' discs compared to 44% of scoliotic discs ($p=0.013$), and they were more common in the anterior anulus compared to the posterior ($p=0.026$). Maximum penetration of blood vessels from the peripheral anulus was 4.7 mm (in 'painful' discs) and 2.0 mm (in control discs), and penetration increased with histological grade of disc degeneration in the 'painful' discs ($p=0.002$). In 16/17 'painful' discs, blood vessels were within 1 mm of an anulus fissure, or the disc periphery. Nerves were found in 36% of 'painful' discs (all with blood vessels) and 25% of control discs. Nerve ingrowth was always less than or equal to blood vessel ingrowth, with a maximum observed penetration of 1.5 mm from the annulus periphery.

Discussion: In degenerated and painful discs, the ingrowth of nerves appears to follow that of blood vessels, and is facilitated in the region of annulus fissures. No nerves were seen >2mm from the annulus periphery, suggesting that previous reports of nerves in the disc nucleus may refer to vertical growth from a vertebral endplate rather than radial growth through the annulus. Results support the view that discogenic back pain is associated with pain-sensitisation events in the disc periphery.

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P124

A NEW SCORING SYSTEM FOR OSTEOARTHRITIS OF THE KNEE

L Nelson*[1], HE McCarthy[1], J Fairclough[1], CW Archer[1]
Cardiff University, School of Biosciences, Museum Avenue, Cardiff, CF10 3AX. UK

A novel scoring system for the grading of osteoarthritis has been developed.

Scoring systems for the measurement of Osteoarthritis (OA) are essential for the understanding of the osteoarthritic process. OA is a mutifactorial degenerative joint disease affecting not only hyaline cartilage but also the surrounding tissues and particularly the subchondral bone. It as questionable as to why the articular cartilage remains the sole component used for histopathological assessment. The intimate relationship between the subchondral bone and overlying cartilage provide major difficulty in their independent measurement.

A new scoring system has been developed to incorporate the subchondral bone into the assessment process and relating it to the structure of the overlying hyaline cartilage, which together permit a more accurate description of the degree of degenerate change.

The new scoring system was developed from the analysis of 26 operative specimens from tibial plateau (TP) from patients who underwent total knee replacement (TKR). Multiple osteochondral plugs were taken from weight-bearing regions of the whole TP. The specimens were fixed and decalcified before being sectioned and stained with Masson's trichrome.

Using a standard imaging system (Photoshop) the areas of bone and hyaline cartilage were identified and measured. Further parameters 1) cartilage thickness 2) tidemark integrity, 3) surface integrity 4) cartilage morphology were measured using a numeric measurement scale.

The scoring system indicated a relationship between the area of subchondral bone and the hyaline cartilage degeneration. The overall sum of scores was also successful in distinguishing between the milder and more severe samples of OA. More comprehensive inter and intra observer variability needs to be tested in order validate the system. Quantifying changes to the subchondral bone may also serve beneficial to clinicians, as it is possible that monitoring these changes clinically could lead to early identification of OA.

P125

AN OSTEOCHONDRAL CULTURE MODEL SYSTEM FOR THE STUDY OF ALLOGENIC ARTICULAR CARTILAGE DERIVED PROGENITOR CELLS IN CARTILAGE REPAIR

A Chandrashekran*[1], J Kelly[1], R Williams[2], C Archer[2], A Goodship[1]
[1]Institute of Orthopaedics and Musculoskeletal Sciences, University College London, UK; [2]Cardiff School of Biosciences, Cardiff University, UK

Unique progenitor cells have been identified recently and successfully cultured in vitro from human articular cartilage. These cells are able to maintain chondrogenic potential upon extensive expansion. In this study, we have

developed a sheep, ex-vivo model of cartilage damage and repair, using these progenitor cells. This study addresses the question can such a model be used to determine factors required for progenitor cell proliferation, differentiation and integration of matrix onto bone. The hypothesis was that sheep allogenic cartilage derived progenitor cells could regenerate artificially damaged sheep articular cartilage in an osteochondral culture model. Progenitor cells were derived from ovine articular cartilage using a differential adhesion assay to fibronectin and expanded clonally. These clonal cells were marked with lentiviral vectors derived from the Human Immunodeficiency Virus-1. When a self-inactivating lentiviral vector encoding a ubiquitous phosphoglycerate kinase promoter, driving a Green Fluorescent Protein (GFP) reporter gene, was used to transduce these cells, up to 80% of these progenitor cells expressed GFP. Normal sheep medial femoral condyles containing about 2mm thick sub-condral bone were obtained and 4mm circular defects created on the cartilage surface using a biopsy punch. Condyles were cultured for two weeks in vitro with GFP labelled progenitor cells within a fibrin glue scaffold (Tisseel Lyo) and matrix production (collagen) as determined by spatially offset Raman spectroscopy and immunohistochemistry was demonstrated. Progenitor cells were able to proliferate and differentiate into collagen producing cells. Such an ex-vivo model system is an effective tool for the analysis of cartilage repair from various sources of stem cells. These ex-vivo experiments and variations on defect type, size, titration of scaffold and progenitor cell numbers requirements can further be used as a basis for screening prior to in vivo experiments.

P126

FRACTURE MECHANICS VALUES FROM CANCELLOUS BONE FROM THE HEAD OF THE FEMUR: RELATIONSHIPS TO NON-INVASIVE SKELETAL ASSESSMENT MEASUREMENTS

RB Cook[1], C Curwen[2], T Tasker[2], P Zioupos*[3]
[1]nCATS, School of Engineering Sciences, University of Southampton, UK; [2]Dept of Trauma & Orthopaedics, Gloucestershire Royal Hospital, Gloucester, UK; [3]Biomechanics Laboratories, Cranfield University, Shrivenham, UK

Osteoporosis (OP) results in a reduction in the mechanical competence of the bone tissue of the sufferers. In skeletal sites such as the proximal femur and the vertebrae, OP manifests itself in low trauma fragility fractures which are debilitating for the patient. The relationships between the compressive strength of cancellous tissue and its apparent density are well established in studies of the past. Recently the authors have presented a method able to assess the fracture toughness properties of cancellous bone (1), a challenging cellular material which can exhibit large elasto-plastic deformations. The in-vitro measurement of fracture toughness alongside the customary compressive strength can provide a comprehensive assessment of the mechanical capacity of cancellous bone, which will reflect closer its ability to resist crack initiation. The aims of the present study were: (1) to examine whether the observed fracture toughness deterioration can also be detected by non-invasive quantitative ultrasound (QUS); and (2) to provide rational evidence for the well proven ability of QUS to predict directly 'risk of fracture'. 20 femoral heads were obtained from donors undergoing emergency surgery for a fractured neck of femur. QUS investigations of the calcaneus, proximal phalanx and distal radius were undertaken within 72 hours of surgery. 128 fracture toughness samples and 20 compression cores were manufactured and tested. Two clinical QUS systems were used to obtain in-vivo scan data and then directly compared those to the density, porosity and the fracture mechanics of tissue extracted from the same individuals. The results demonstrated not only that there was a significant link between in-vivo determined QUS values for the calcaneus and finger to the density of the density of the femoral head; but that there was also a significant link between the QUS results from the calcaneus and the fracture toughness of the cancellous bone from the femoral head. These results point towards a systemic effect of osteoporosis which affects similarly different parts of the skeleton and supports the use of clinical QUS systems as a diagnostic tool for the prediction of fracture risk.

Refs: (1) R.B. Cook, P. Zioupos. 2009. J Biomech. 42, 2054-2060.

P127

BONE AT HIGH STRAIN RATES: AGE, DUCTILE TO BRITTLE TRANSITION AND BIOPHYSICAL EFFECTS

P Zioupos*[1], HOK Kirchner[2], S Puchegger[3]; H Peterlik[3]
[1]Biomechanics Laboratories, Cranfield University, Shrivenham, UK; [2]UMR8182, Universite Paris-Sud, F-91405 Orsay, FR; [3]Faculty of Physics, University of Vienna, A-1090 Vienna, Austria

Human bone becomes increasingly brittle with ageing. However, traumatic failures in-vivo are more likely to be orders of magnitude faster than the quasistatic tests usually employed in-vitro, and in fact bone fractures differently under slow and fast loadings, being ductile and brittle, respectively. We have reported recently (1) results from tests on specimens of human femoral cortical bone loaded in tension at strain rates ranging from low (0.08/s) to high (18/s). At high strain rates bones were brittle; at low strain rates they were much tougher. A post-test examination of the microcracking damage (2) revealed that microcracking was inversely related to the strain rate with specimens loaded at low strain rates showing considerable post-yield strain and more microcracking. Partial correlation and regression analysis suggested that the development of post-yield strain (effect) was a function of the amount of microcracking incurred (the cause), rather than being a direct result of the strain rate (the excitation). Presumably low strain rates allow time for microcracking to develop, which increases the compliance of the specimen, making them tougher. This behaviour confirms a more general rule that the degree to which bone is brittle or tough depends on the amount of microcracking damage it is able to sustain. The key to bone's toughness is its ability to avoid a ductile-to-brittle transition (DBT) for as long as possible. The key to bone's brittleness is the strain and damage localization early on in the process, which leads to low post-yield strains and low energy absorption to failure. When strain rate was combined with ageing there was a shift of the DBT threshold to lower strain rates. Other complementary biophysical tests by using X-ray diffraction and a synchrotron source, have linked this behaviour to the basic physical behaviour of the collagen that comprises 95% of the organic bone matrix, thus providing a rationalistic basis for understanding these, otherwise, stochastic events in the fracture of bone.

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P128

HIGH TIBIAL OSTEOTOMY: LINKING BIOMECHANICAL AND BIOLOGICAL CHANGES

GM Whatling[1], CA Holt*[1], K Brakspear[2], H Roberts[2], D Watling[1], RS Kotwal[3], C Wilson[3], R Williams[3], A Metcalfe[3], J Sultan[3], DJ Mason*[2]
[1]School of Engineering, Cardiff University, Cardiff, UK; [2]School of Biosciences, Cardiff University, Cardiff, UK; [3]University of Wales Hospital Llandough, Cardiff; [1,2,3]Arthritis Research UK Biomechanics and Bioengineering Centre, Cardiff UK

High tibial Osteotomy (HTO) realigns the forces in the knee to slow the progression of osteoarthritis. This study relates the changes in knee joint biomechanics during level gait to glutamate signalling in the subchondral bone of patients pre and post HTO. Glutamate transmits mechanical signals in bone and activates glutamate receptors to influence inflammation, degeneration and nociception in arthritic joints. Thus glutamate signalling is a mechanism whereby mechanical load can directly modulate joint pathology and pain.

3D motion analysis was used to assess level gait prior to HTO (n=5) and postoperatively (n=2). A biomechanical model of each subject was created in Visual3D (C-motion. Inc) and used for biomechanical analysis. Gene expression was analysed by RT-PCR from bone cores from anterior and posterior drill holes, subdivided according to medial or lateral proximal tibia from HTO patients (n=5).

Knee adduction moment is a clinical marker of medial compartment loading. Pre-operatively the mean peak adduction moment was 3.8 ± 1.8 % body weight times height (BW.h). One subject maintained a consistent peak adduction moment pre (1.8 %BW.h) and post-operatively (1.9 %BW.h) with a reduction in the second moment peak. Another subjects peak adduction moment was significantly reduced from 6.7 %BW.h pre-operatively to 1.4 %BW.h postoperatively. GAPDH, osteocalcin, EAAT-1, EAAT1ex9skip, NR2A, KA1, OPG and RANKL mRNA expression was detected in HTO bone cores. In one patient, where HTO reduced medial compartment loading, differential expression of EAAT1ex9skip and KA1 was observed in pre and post HTO bone cores.

Changes in knee adduction moments following HTO have been identified indicating altered medial compartmental loading. This is being investigated further in larger cohorts in a 5 year study. We have demonstrated that glutamate transporters and receptors are expressed in human subchondral bone and that glutamate transporter mRNA expression may vary after HTO surgery. In arthritis, glutamate concentrations in the synovial fluid are increased, activating receptors in joint tissues and nerves to influence pathology and nociception. Thus glutamatergic signals represent a direct mechanism linking mechanical loading through the joint to pathology and pain in human arthritis.

P129

AGED AND DEGENERATIVE HUMAN INTERVERTEBRAL DISCS: IS THERE A DIFFERENCE?

MM Al-Abbasi*[1,2] MA Adams[1], JF Tarlton[2]
[1]Centre for Comparative and Clinical Anatomy, University of Bristol; [2]Matrix Biology Research Group, University of Bristol, UK

It is a challenge to distinguish intervertebral disc degeneration from normal age-related changes. However, severely degenerated discs exhibit regions that are clearly pathological, being physically disrupted and biochemically altered. Therefore we compared biochemical composition in such abnormal regions with composition in less severely affected regions of the same discs, in order to gain insights into the degenerative process beyond age related changes.

Whole discs were removed from 102 elderly cadaveric spines (T9-S1) with a median age of 81yr. Each disc was dissected to yield samples of anterior annulus (AAF), nucleus pulposus (NP) and posterior annulus (PAF). Samples were separated into areas with evident pathological changes (i.e. Disrupted), and areas with reduced or no changes compared to other discs of similar age (Non-Disrupted). Collagen, sulphated-glycosaminoglycans (sGAG) and water content were measured, using hydroxyproline and Farndale assays respectively.

Collagen content was the greatest in the PAF (386.5± 135.3) and the least in the NP (182.1± 68.45) (P<0.001). Unusually, GAG and water content were similar across all regions in these old discs in the absence of disruption. Importantly, water and sGAG content was significantly lower in the disrupted parts of each disc region, compared to the non-disrupted parts (P<0.01). Collagen content was also reduced in the disrupted parts of the NP (123.3± 86.23), and PAF (282.9± 67.88) (P<0.001).
sGAG and water levels may be similar in the nucleus and anulus because these old discs no longer had a hydrostatic nucleus, so that all tissues were subjected to direct compressive loading. Losses in collagen, sGAG and water content are specifically related to degeneration when comparing age matched (i.e. same patient) samples, which may reflect swelling and leaching following collagen damage. This may lead to a cycle of destruction in chronic disease, consistent with physical disruption and altered function.

P130
IN VITRO CULTURE OF OSTEOBLASTS WITHOUT THE USE OF ANIMAL DERIVED MATERIAL
C Elford[1], DJ Mason[2], J Ralphs[2], JW Gregory[1], AJ Sloan[3], BAJ Evans[1]*
[1]Department of Child Health, School of Medicine, Cardiff University; [2]Division of Pathophysiology and Repair, School of Biosciences, Cardiff University; [3]Tissue Engineering and Reparative Dentistry, School of Dentistry, Cardiff University, UK
It is important to advance and implement the 3Rs i.e. replacing, reducing and refining the use of animals in research and testing. Furthermore, musculoskeletal cell or tissue replacement therapies require the use of chemically defined media, which do not contain animal derived materials. We investigated the use of a range of non-FCS containing media on cell number (3 days; MTS assay) in human osteoblast lines (MG63, SaOS2). We have compared the current gold standard of DMEM containing 10% FCS with a range of test media: 1) DMEM with 10% human serum (Lonza), 2) DMEM with 10% human serum AB (Lonza) 3) DMEM with K/O serum replacement (10%, 15%; Invitrogen), and 4) TheraPEAK (defined medium; Lonza). We also investigated the effects of these media on multipotent, marrow-derived, human mesenchymal stem cell (MSC; Promocell) numbers and differentiation (3 days; ALP activity). Cells were either i) set up in control media and test media added the next day, or ii) set up in the test media.
With MG63 cells, human serum and K/O decreased (50-60%; p<0.001), whereas TheraPEAK increased numbers (~130%; p<0.001). With the more differentiated SaOS2, however, human serum increased (120%; p<0.01) or had no effect, depending on the set up methodology used, K/O (15%) decreased (~35%; p<0.01), whereas TheraPEAK increased numbers by 200 to 300% (p<0.001). With undifferentiated MSCs, results were similar to those with MG63 cells, although reductions with human serum and K/O were ~25% and ~75% respectively (p<0.001). Human serum and TheraPEAK stimulated (~250%; p<0.001) differentiation (ALP activity) in MSCs. Human bone chips set up in different media resulted in the establishment of osteoblast cultures in all media, although FCS containing cultures exhibited most proliferation, whereas osteoblasts in human serum containing medium were the most differentiated. Very few cells were observed with TheraPEAK.
The work shows that cells at different stages of the osteoblast differentiation pathway respond differently to non-FCS containing media, and that it is possible to establish primary, human osteoblast cultures without using FCS. The results will guide our planned studies to design successful osteoblast and osteocyte culture methods using media which do not contain animal derived materials.

P131
OSSEOINTEGRATION OF SILVER TREATED TITIANUM ALLOY
MJ Coathup*[1], J Shawcross[2], C Scarsbrook[1], M Korda[1], A Hanoun[1], M Pickford[2], P Agg[2], GW Blunn[1]
[1]John Scales Centre for Biomedical Engineering, Institute of Orthopaedics and Musculoskeletal Science, Division of Surgery and Interventional Science, University College London; [2]Accentus Medical, Harwell Science and Innovation Campus, Didcot, Oxfordshire, UK
Introduction. A modified anodisation technique where a titanium surface releases bactericidal concentrations of silver was developed and called Agluna. Our hypothesis was that silver incorporation was bactericidal and had no effects on the viability of fibroblasts and osteoblasts, would have no negative effect on interfacial shear strength and bone contact in an in vivo trans-cortical implant ovine model.
Methods. In vitro: Titanium alloy discs were either polished (Ti), anodised (Ano), anodised or Agluna treated (Ag) or anodised and Agluna treated followed by a conditioning step (Ag C). Conditioning was achieved by incubating discs in culture fluid for 48 hrs. The bactericidal effect of these discs was tested by measuring the zone of inhibition of different bacteria grown on agar. Live/dead staining was carried out and silver levels measured using atomic emission spectroscopy. 8 implants were inserted into each sheep (60 in total (n=5)). Grit blasted Titanium alloy (Gb) and Agluna treated grit blasted titanium alloy (Ag) at a silver concentration of 4-6 micrograms/cm2 were compared at 6 weeks. Gb implants, Ag (at 4-6micrograms/cm2), high dose Agluna implants with silver concentrations at 15-20micrograms/cm2 (HdAg) and a grit blasted anodised titanium alloy (Ano) were compared at 12 weeks. Pullout strength and bone-implant contact was quantified.
Results. On Ti, Ano and Ag C surfaces the number of live fibroblasts was significantly greater than on Ag (non-conditioned) surfaces. Data from pull out tests at 6 weeks showed a lower but significant interfacial shear strength in the Ag group (310.4N) when compared with the Gb group (561.2N) (p=0.01). At 12 weeks, there were no significant differences between each of the 4 treatment groups. Histological analysis showed no significant differences in bone-implant contact between groups at 6 and 12 weeks.
Discussion. The initial non-conditioned Agluna surface is bactericidal and cytotoxic but on conditioning, osteoblasts and fibroblasts attached and remained viable. The condition Agluna surface remains bactericidal. Silver incorporation at a concentration up to 20 micrograms/cm2 has no adverse toxic effect on osteointegration and the interfacial shear strength of implants. This coating has been used clinically in situations where the infection rate is high.

P132
CHARCOT NEUROPATHIC OSTEOARTHROPATHY, PRO-INFLAMMATORY CYTOKINES AND BONE TURNOVER MARKERS
RG Pearson*[1], KSS Shu[1], H Divyateja[2], M Seagrave[1], FL Game[2],WJ Jeffcoate[2], BE Scammell[1]
[1]Department of Orthopaedic & Accident Surgery, University of Nottingham, Queen's Medical Centre, Nottingham. NG7 2UH, UK; [2]Department of Diabetes and Endocrinology, Nottingham University Hospitals Trust, Nottingham, UK
Background: Charcot neuropathic osteoarthropathy is a rare, destructive process affecting the bones and joints of feet in patients with diabetic peripheral neuropathy. The aetiology of Charcot remains unknown, although it has been suggested that it is triggered by the occurrence of inflammation in the foot of a susceptible individual, and that the inflammation results in increased osteoclastic activity.
Hypothesis: The increased bone turnover in acute Charcot is associated with increased concentrations of pro-inflammatory cytokines, related signalling peptides and bone turnover markers.
Methods: 17 patients newly presenting with acute Charcot in diabetes and 16 non-diabetic patients without neuropathy undergoing elective forefoot surgery provided informed consented to participate. Samples of bone were taken by needle biopsy, and were stained with H&E to determine bone architecture and bone remodelling. Serum ALP, CTX, OPG and sRANKL TNF, IL1-beta, IL6 and CRP were measured by immunoassay. Blood was taken from the dorsal foot vein of both the affected and the unaffected foot, as well as an antecubital vein.
Results: Classic histopathology features of fracture and bone remodelling were evident in Charcot bone biopsies. Systemic circulating concentrations in the Charcot group antecubital vein for both IL6 and OPG were significantly greater than in controls (p<0.05). There were no significant differences between the dorsal vein concentrations of any analyte when the affected and unaffected feet were compared. However, in patients with an acute Charcot foot the concentration of OPG, ALP and CTX was higher in sera from the dorsal vein of affected foot when compared to controls (p<0.05), this difference was highly significant for IL6 (p<0.001).
Conclusion: The elevation in CTX observed in the affected foot in patients with an acute Charcot foot reflects the bone breakdown and remodelling which is present. The higher circulating concentration of IL-6 in the Charcot patient group, reflects the inflammation which is present and which is thought to be central to the development of the condition. Although OPG values were significantly greater in Charcot than control group, circulating concentrations of OPG are known to be higher in diabetes.

P133
CAN RAMAN SPECTROSCOPY BE USED TO MEASURE QUALITATIVE DIFFERENCES IN THE ORGANIC PHASE OF BONES WITH VERY DIFFERENT MINERAL VOLUME FRACTIONS?
K Buckley*[1], P Matousek[1], AW Parker[1], AE Goodship[2]
[1]Central Laser Facility, STFC Rutherford Appleton Laboratory, Harwell Oxford, UK; [2]UCL Institute of Orthopaedics and Musculoskeletal Science, Royal National Orthopaedic Hospital, Stanmore, UK
The chemical composition, and hence the mechanical properties, of bone can vary according to its function. The biochemical mechanisms which control this compositional variation are not well understood but we hypothesize that it is guided by the some feature of the collagen chemistry. Raman spectroscopy is an analytical technique which uses the inelastically scattered photons to probe chemical composition. It can be applied to measure relative proportions of hydroxyapatite and collagen in different bony materials, and we hypothesize it can be used to elucidate what determines it. The elucidation of the control mechanism, apart from being interesting from a biochemical point of view, could provide therapeutic targets for the management of bone conditions i.e. enabling the control of the mechanical properties of bone. In this study we inspect the Raman spectra of various functionally adapted bones with very different mineral to collagen ratios. These include bones with relatively large proportions of mineral from the ear (e.g. fin whale tympanic bulla) adapted to be very stiff and bones with relatively large proportions of collagen (e.g. red deer antler). The interesting result is the different bones have differences in the

profiles of the Raman spectra of the collagen bands as well as in their intensities. The next step of the research will be to deduce what feature of the collagen chemistry these Raman profile changes represent, and to establish the causal relationship from the spectra to mechanical properties.

P134

SPATIALLY OFFSET RAMAN SPECTROSCOPY: A NOVEL METHOD FOR THE DETECTION OF MOLECULAR CHANGES IN HUMAN KNEE OSTEOARTHRITIS

JG Kelly*[1], PD Gikas[1,2], JR Perera[1,2],K Buckley [1,3], A Shepperd[1], HL Birch[1], J Miles[2], TWR Briggs[2], AW Parker[3], P Matousek[3], AE Goodship[1]
[1]Institute of Orthopaedics and Musculoskeletal Science, UCL, Royal National Orthopaedic Hospital, Stanmore, Middlesex, UK; [2]Royal National Orthopaedic Hospital, Stanmore, Middlesex, UK; [3]Central Laser Facility, STFC Rutherford Appleton Laboratory, Didcot, Oxfordshire, UK

Osteoarthritis is a common, debilitating disease of joints involving degeneration of cartilage and bone. Subtle changes in the molecular structure of subchondral bone precede morphological changes in the osteoarthritic joint.

Raman spectroscopy measures inelastic scattered laser light produced when photons interact with chemical materials. Resultant changes in wavelength form spectra representative of the chemical composition of the given sample. Using the new Raman technology of spatially offset Raman spectroscopy (SORS) it is possible to obtain chemical composition of materials several centimetres beneath a surface. Therefore SORS has the potential to be usefully employed in a clinical context to measure bone beneath cartilage. The aim of our study is to explore the hypothesis that abnormal molecular changes in subchondral bone can be detected with SORS. This extends previous biological work that has shown a high proportion of abnormal homotrimeric collagen in subchondral bone from osteoarthritic hip joints.

Raman spectra were acquired ex vivo from 20 human tibial plateaus with established medial compartment osteoarthritis (radiographic and macroscopic diagnosis). Spectra were analysed to determine the spectral peak height ratio of carbonate-to-phosphate (indicating degree of carbonate substitution in the hydroxyapatite crystals) and phosphate-to-collagen (a measure of mineral/organic ratio). Spectral analyses were compared with biochemical analysis (collagen I alpha chain ratios). A peripheral quantitative computed tomography (pQCT) was used to measure the bone mineral density (BMD) across the samples.

Our spectral data will be presented comparing the medial and lateral sides of the tibial plateau. pQCT results revealed that the subchondral bone of the medial side of the samples was both denser and thicker than that of the lateral side.

The immediate goal is to provide an early indication of joint damage, prior to clinical observations, based on correlating molecular-specific modifications in the subchondral bone. Ultimately our efforts will seek to assess SORS for both characterising and detecting osteoarthritis during its early subclinical phase.

P135

IDENTIFICATION OF AN EXTRAORDINARY BONE PHENOTYPE IN A PATIENT WITH ALKAPTONURIA

AM Taylor*[1], JS Davidson[2], JC Jarvis[1], LR Ranganath[1], JA Gallagher[1], A Boyde[3]
[1]Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK; [2]Department of Trauma & Orthopaedics, Royal Liverpool and Broadgreen University Hospitals Trust, Liverpool, UK; [3]Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

A 59 year old man with known alkaptonuria (AKU) presented for surgery due to ochronotic osteoarthropathy and underwent a total hip replacement. The resected femoral head showed uniform ochronosis of the articular cartilage with the presence of a large osteophyte on the neck of femur, also demonstrating ochronosis. Surrounding capsular tissues displayed patchy ochronotic pigmentation. An ultrastructural and histological analysis of patient tissues was undertaken. Samples were processed for histology and for topographical 3D scanning electron microscopy (SEM) and quantitative back scattered electron SEM (qBSE-SEM). These analyses revealed an extraordinary phenotype including the presence of some novel microanatomical structures. Histological examination of the cartilage demonstrated the presence of ochronotic pigment throughout all zones of articular cartilage, both intra- and extracellularly. No similar pigmentation was observed in the calcified cartilage or bone matrices, but these latter tissues underwent significant structural modelling. Ochronosis was observed in osteoblasts, osteoclasts and osteocytes, with osteoclasts seen engulfing pigmented osteocytes from the bone matrix. The most striking feature was the resorption of subchondral bone and calcified cartilage such that in advanced stages there was complete loss of the subchondral bone plus calcified cartilage plate. The underlying trabecular bone also contained idiosyncratic architecture. Trabecular surfaces had numerous outgrowths that we have termed 'excrescences' of which three distinct types were recognized. The first type arose from the incomplete resorption of branching trabeculae and they were characterized by scalloped surfaces and rugged edges. The second type arose in a similar way but had been smoothed over by new bone deposition. The third type which resembled coarse stucco probably arose from resting surfaces that had been focally reactivated. These were poorly cemented to the prior trabecular wall. They had a disorganized arrangement of collagen fibres and contained poorly mineralised and hypermineralised regions. We propose these distinctive microanatomical structures do not arise as a result of pigmentation per se but because of abnormal osteoclast/osteoblast modelling secondary to altered mechanical loading or other aberrant signalling. The distinctive subchondral environment underneath pigmented cartilage in AKU provides a unique model to investigate the response of bone to altered mechanical loading.

P136

TRABECULAR EXCRESCENCES ARE PRESENT IN OSTEOARTHROPATHIES INCLUDING OSTEOARTHRITIS

AM Taylor*[1], A Boyde[2], JS Davidson[3], JC Jarvis[1], LR Ranganath[1], JA Gallagher[1]
[1]Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK; [2]Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; [3]Department of Trauma & Orthopaedics, Royal Liverpool and Broadgreen University Hospitals Trust, Liverpool, UK

We recently discovered some novel microanatomical structures, which we termed trabecular excrescences (TEs), in bone in patients with the severe osteoarthropathy of alkaptonuria (AKU). We wanted to determine if these structures were specific to AKU bone, and therefore we undertook an extensive survey of non-AKU bone. We now report that TEs are present in other osteoarthropathies including osteoarthritis. Tissues were processed for histology, scanning electron microscopy (SEM), topographical, quantitative back scattered electron SEM (qBSE-SEM) and 3-Dimensional SEM. Using these imaging techniques, we were able to detect several different morphologies of TEs in trabecular bone. One type arose from the incomplete resorption of secondary trabeculae projecting above the general plane of the local bone surface. These are characterised by deeply scalloped surfaces and rugged edges and resorption involved here is deeper and shows larger pits or scoops than those of normal resorption fields. The second appearance is similar to the first but they have been smoothed over by new bone deposition. A third resembles coarse stucco and seems to have arisen where resting surfaces have been focally reactivated. These appear as random patches on the bone surface. These deposits are frequently poorly attached to the prior trabecular wall as demonstrated by clearly observable discontinuities between the excrescences and the otherwise continuous trabecular surfaces. Over the past few years the concept of resorption-formation coupled remodelling has become a dominant paradigm in bone turnover. Whilst formation of TEs involves both osteoblastic and osteoclastic activity, they do not arise though classical coupling. The discovery that TEs are present in OA and are not restricted to AKU bone raises the question of how widespread is their occurrence in human bone and whether or not they are only found in pathologies. We detected them in 12/12 samples of AKU bone and 7/10 OA cases investigated. It is interesting to note that although TEs are readily detectable by conventional histology and by SEM, it is unlikely that they would be detected by routine micro CT. This research underlines how studying rare disease of bone can lead to advances in understanding more common disorders of the skeleton.

P137

ROENTGENOLOGYCAL STUDY OF CALCANEONAVICULAR JOINT

VI Luzin, VN Voloshin*, IS Voloshina
Department of Human Anatomy, State Medical University of Lugansk, Ukraine

Calcaneonavicular joint is defined as abnormal coalescence of the calcaneus with the tarsal navicular bone. The normal morphologic relationship of the calcaneus with the navicular bone can be described as a slender gap between the two articulated bone structures. The scientific literature about calcaneonavicular morphology has been based primarily on retrospective findings of association between radiographically or surgically proved calcaneonavicular coalition in particular patients and a previously defined clinical syndrome in those patients. The purpose of the present study was to determine radiographically demonstrated variations in calcaneonavicular morphology.

Variations in calcaneonavicular morphology depicted on the medial oblique view were classified into four groups according to morphologic type, and the prevalence of each type was calculated. We compared the prevalence of each type in male and in female cadavers. One-way analyses of variance were used to compare mean ages of patients for each type and mean calcaneonavicular gaps for each type.

94 from 105 variations (89.52%) were characterized by a wide calcaneonavicular gap and smooth, rounded, and well-defined calcaneal and navicular cortices (first morphological type). Joint produced by synchondrosis (second morphological type) (6.74%) were characterized by a narrow calcaneonavicular gap, flattening and widening of the calcaneus where it approaches the navicular, and smooth, regular, and well-defined cortical surfaces. Joint produced by syndesmosis (third morphological type) (3.74%) were characterized by a narrow calcaneonavicular gap, flattening and widening of the calcaneus where it approaches the navicular, and rough, irregular, and poorly defined calcaneal and navicular cortices. There were no cadavers with type 4 morphology (synostosis). The combined prevalence of types 2 and 3 was 10.48%.

The numbers of male and female cadavers with all morphologycal types were approximately equal (P = 0.876), and there was no statistically significant correlation between any of these three morphologic types and age (P = 0.334). The calcaneonavicular gap was significantly narrower in types 2 and 3 than in type 1 (P = 0.01), which was characterized as the normal morphology.

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ACUTE RESPONSE TO ORAL CALCIUM LOADING IN PREGNANT AND LACTATING WOMEN WITH A LOW CALCIUM INTAKE

I Schoenmakers*[1], GR Goldberg[1,2], K Tsoi[1], LMA Jarjou[2], A Prentice[1,2]
[1]MRC Human Nutrition Research, Cambridge, UK; [2]MRC Keneba, The Gambia

In The Gambia calcium (Ca) intake is low (~350mg/d) and parathyroid hormone (PTH) concentration is elevated throughout life. In pregnancy and early lactation PTH concentration may be suppressed but uncertainty exists about how Ca metabolism is regulated, particularly in populations with low Ca intakes. This pilot study aimed to obtain mechanistic insights. Groups of 10 pregnant (P, 32.6±1.6 wks gestation), lactating (L, 3.13±0.06 mo postpartum), non-pregnant, non-lactating (NPNL) women were given an oral dose of 1000 mg elemental Ca (CaCO3) after an overnight fast. A small standardised meal low in Ca, P and phytates was given at 30 minutes post-dose and 200ml water was drunk every hour. Urine was collected pre-dose and at 2 and 4 hours (h). Blood samples were collected pre-dose and at 3h post-dose. Urinary calcium (uCa), phosphate (uP), creatinine (uCr) and blood ionised calcium (piCa), plasma total Ca (ptCa), phosphate (pP), pPTH, 1,25OH2vitamin D (p1,25OH2D), creatinine (pCr) and albumin (pAlb) were analysed. Within and between group differences were analysed with ANOVA with Scheffé post-hoc tests as appropriate. The level of significance was set at $P<0.05$.

Baseline plasma concentrations of ptCa, pAlb and pCr were significantly lower and p1,25OH2D higher in P women than in L and NPNL women. In P women pPTH was lower than in L women. There were no differences between P women and L and NPNL women in piCa, pP, uCa/Cr and uP/Cr.

Post loading, plasma concentrations of piCa and ptCa increased and pPTH decreased in all groups. Only in P women pP decreased and p1,25OHD significantly increased. In all groups uCa/Cr significantly increased and uP/Cr decreased from 2 to 4h post loading. The magnitude of change did not differ between groups for any of the analytes in blood and urine.

Conclusion: In pregnant women with a low calcium intake pPTH is suppressed and p1,25OH2D is elevated. P, L and NPNL women respond to a similar extent to Ca-loading, suggesting that the parathyroid gland responds similarly to changes in piCa. These findings differ from those in pregnant and lactating women with a Ca intake close to the Western recommendations (Kent et al 1991).

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INSTRUMENTED TIBIAL NAILS FOR MONITORING NAIL-BONE LOAD SHARING DURING FRACTURE HEALING

SJG Taylor*[1], W Mahmood[1], R Faroug[1], I McCarthy[1], D Wilson[2]
[1]Institute of Orthopaedics and Musculoskeletal Science, UCL, UK; [2]Smith & Nephew Research Centre, York, UK

Early diagnosis of delayed- and non-union tibial fractures is difficult, but treatment options are available if timely data are available. Direct correlation between implant forces and healing status is difficult during stance phase loading due to soft tissue forces. This ongoing study seeks to find a minimal set of strain gauge sites needed to determine healing at any of several fracture sites, using isometric loading suitable for routine clinical usage. A series of instrumented tibial nails are being used to help determine whether an alternative technology can replace or augment existing routine methods for assessment of fracture healing.

In a prior study, a single strain gauge positioned close to the fracture site had produced mixed results. In the current study, a TRIGEN META NAIL, 10mm OD x 380mm long, was instrumented with 8 gauged sites spiraled down the nail at 34mm axial and 120deg angular separation (Gen1), and loaded in a Sawbone model in offset axial compression, 3 point bending and torque.

In order to gain early clinical results, and in a design informed by the Gen1 data, a set of instrumented nails have been made for an ovine wireless telemetry study (Gen3a), shortly to commence, in which the tibial nail has been over-gauged enabling multiple d.o.f. measurements to be made during gait, torque, axial compression and 3 point bending; the latter protocols offering more controlled patient postures. This study is to be followed by a similar human study (Gen3) involving five subjects (12 gauges per nail). Meanwhile, a parallel biomechanical study involving six nails with 20 gauges each is also planned.

In the Gen1 study, the strains diminished with distance from the fracture site and with out-of-plane sites during bending. During torque, however, the response was much more uniform for all strain sites. Significant increases in strains due to both loading regimes were seen in the fractured case vs. an intact bone.

Preliminary conclusions are that strains measured due to applied torque may offer a more sensitive and fracture site-independent means of assessing healing than induced bending. We now aim to confirm these observations in animal and human studies.

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FRICTION IN METAL-ON-POLYETHYLENE LUMBAR TOTAL DISC REPLACEMENTS

PJ Hyde*, JF Fisher, RM Hall
Institute of Medical and Biological Engineering, University of Leeds, Leeds, UK

Spinal total disc replacement (TDR) designs rely heavily on total hip replacement (THR) technology and it is therefore prudent to check that typical TDR devices have acceptable friction and torque behaviour. For spherical devices friction factor (f) is used in place of friction coefficient (mju). The range of loading for the lumbar spinal discs is estimated at perhaps 3 times body weight (BW) for normal activity rising to up to 6 times BW for strenuous activity [1]. For walking this equates to around 2000 N, which is the maximum load required by the ISO standard for TDR wear testing [2].

Three Prodisc-L TDR devices (Synthes Spine) were tested in a single station friction simulator. Bovine serum diluted to 25% was used as a lubricating medium. Flexion-extension was ±5 deg for all experiments with constant axial loading of 500, 2000 and 3000 N. The cycle run length was limited to 100 and the f and torque (T) values recorded around the maximum velocity of the cycle point and averaged over multiple cycles.

Preliminary results shows that the 500 N loading produced the largest f of 0.05 ± 0.004. The 2000 N load, which approximates daily activity, gave f = 0.036 ± 0.05 and the 3000 N load gave f = 0.013 ± 0.003. The trend was for lower f with increasing loads.

A lumbar TDR friction factor of 0.036 for a 2000N load and the reduction in f for increasing loads is comparable to the lower end of the range of values reported for THR in similar simulator studies using metal-on-polyethylene bearing materials [3]. The 3000 N result showing that increasing the load above that expected in daily activity does not raise the f could be important when considering rotational stability and anchorage in a TDR device because frictional torque at the bearing surfaces is proportional to the product of load, device radius and f.

1. JP Callaghan et al., Clinical Biomechanics (1999)
2. ISO-18192-1, Implants for surgery
3. RM Hall and A Unsworth, Biomaterials (1997)

P141

VARIATION IN FEMORAL NECK CORTICAL THICKNESS IN HIP FRACTURE CASES AND CONTROLS: HISTOLOGICAL ANALYSIS OF BIOPSIES FROM 113 SUBJECTS

J Power[1], M Parker[2], H Kroger[3], N Rushton[1], N Loveridge[1], J Reeve*[1]
[1]University Departments of Medicine & Surgery, Cambridge UK; [2]Peterborough General Hospital UK [3]Kuopio University Hospital, Finland

Maintaining femoral neck cortical thickness may help prevent hip fracture. Fracture initiation probably starts superiorly at flaws, ie where the cortex is thinnest. Whole body computed tomography (QCT) is now being used to study cortical thickness but limited resolution (> 300 micrometers) makes in vivo estimates imprecise, whereas microscopy s resolution approaches 1 micrometer. We have therefore extended our microscopic studies on femoral neck biopsies to include men (14 cases, 26 controls) and women (50 cases, 23 controls), and here provide data on true cortical thickness in subjects with and without hip fracture.

Whole femoral neck cross-sections obtained at hemiarthroplasty (or at post-mortem in controls) were embedded in methacrylate, cut, stained and imaged at medium power. Image-J was used to define cortical boundaries and to measure cortical thicknesses at 5 degree intervals of arc from the cross-sections centre of area.

We confirmed that the mid-femoral neck (or narrow neck) site, defined as where the ratio of maximum to minimum neck diameter (max:min) is 1.4, shows great asymmetry, with the thick inferior cortical octant averaging over 3mm thickness (mean age 79 years inter-quartile range 74-85). In the superior 3 octants cortical thickness averaged 26% of that seen inferiorly. To assess statistical determinants of cortical thickness, the data were modelled with linear regression in octants after adjusting for subjects age, sex, max:min, and hip fracture status. To achieve normality of residuals the cortical thickness data were log-transformed. 95% of measured cortical thicknesses fell between 45% and 220% of the mean for octant. In the thinner, superior three octants, minimum thicknesses were just under 0.3 mm in the fracture cases ie close to 35% of the subjects mean for octant. Cases had about 17% thinner cortical thicknesses in all octants than controls, while female controls had cortical thicknesses that uniformly averaged 90% of male. In conclusion, compared to gender and age-matched controls, intra-capsular hip facture cases had generalized cortical thinning in all mid-neck octants. This disease effect contrasts markedly with the effect of normal ageing, which thins preferentially the mechanically under-loaded superior cortex and spares the infero-anterior cortex.

P142

FIVE YEAR MIGRATION CHARACTERISTICS OF FOUR CEMENTED FEMORAL STEM DESIGNS IN A RANDOMISED TRIAL

MR Downing*, JD Hutchison, GP Ashcroft
[School of Medicine & Dentistry, University of Aberdeen, UK

Prosthesis migration and acetabular cup wear are useful short term measurement which may predict later implant outcome. However, the significance of the magnitude and pattern of the migration is very much dependent on the specific design studied. This study aimed to characterise patterns of migration by following four cemented femoral stem designs using Radiostereometry (RSA) within a prospective randomised longitudinal trial.
164 patients undergoing cemented femoral hip replacement for osteoarthritis were randomised to receive either an Exeter (Howmedica Stryker), Ultima Tapered Polished Stem (TPS) (Depuy), Ultima Straight Stem (USS) (Johnson and Johnson) or Elite Plus (Depuy) stem. Each subject received the OGEE PE cemented acetabular component (Depuy). RSA examinations were performed at 1 week and 6, 12, 18, 24 and 60 months post surgery. They were analysed using the UMRSA system (RSA Biomedical AB, Umea, Sweden), and our local geometric stem measurement software. 149 patients had RSA measurements available to 2 years, and 96 patients to 5 years. Differences were analysed using mixed linear modelling (SPSS).
Median linear proximal cup wear rate reduced to a minimum of 0.02-0.06mm/year in year two. Between 2 and 5 years the wear rate increased, being significantly higher for the Elite.
Cup migration was small but continuous. At 2 years it was median 0.3mm proximally, increasing to 0.5 mm at 5 years. Median rotations were less than 0.3 degrees.
Proximal migration was positive and increasing at all time points for all stems. For the tapered polished designs, while the overall magnitude was significantly higher, the rate of migration significantly decreased, whereas for the other stem designs it did not.
The TPS stem showed a tendency for posterior tilt which was significant compared to the other stems at 5 years.
All stems tended to retroversion, with the USS significantly less than the others and the Elite showing and relative increase at 5 years.
In summary migration patterns are characterised by the stem design, including where there were only small changes between designs. We are now testing measured migrations as predictors of outcome, and will continue to follow this group of patients to 10 years.

P143
BETA-XYLOSIDES INHIBITION OF CHONDROITIN SULPHATE SUBSTITUTION ON MATRIX PROTEOGLYCANS PERTURBS THE DIFFERENTIATION OF BONE MARROW STEM CELLS INTO A CHONDROGENIC LINEAGE
S Li*, B Caterson, AJ Hayes, CE Hughes
Connective Tissue Biology Laboratories, School of Biosciences, Cardiff University, UK
Introduction: Novel chondroitin sulphate (CS) sulphation motifs on cell-associated proteoglycans (PGs) have been shown to be putative biomarkers of progenitor/stem cell sub-populations (Hayes et al., 2007; Dowthwaite et al., 2005). Also, recent studies show that unique CS sulphation motifs are localized in putative stem/progenitor cell niches at sites of incipient articular cartilage & other musculoskeletal tissues (Hayes et al., 2011), which indicates their potential importance in cell differentiation during development. In this study, we investigated the importance of CS in the differentiation of bone marrow stem cells to the chondrogenic phenotype in vitro using p-nitrophenyl xyloside (PNPX) as a competitive inhibitor of CS substitution on matrix PGs.
Methods: Bovine bone marrow stem cells (BMSCs) were isolated from 7-day-old cow hock joints and cultured as monolayer for 4 weeks with chondrogenic medium ± 0.25mM PNPX. DMMB assay, real-time PCR, Western Blotting & immunohistochemistry (IHC) were used to analysis the chondrogenic markers. The expression and distribution of structural CS proteoglycans (CS-PGs) were analysed by immunofluorescent staining combined with confocal microscopy scanning.
Results: BMSCs cultured in chondrogenic medium started to aggregate and form mini-cell beads in 3 days and these mini cell beads clustered together to form a large single alcian blue positive cartilaginous cell bead in 2-4 weeks, indicative of the chondrogenesis. In contrast, there was an apparent delay in the cell bead formation in the BMSCs cultured with PNPX. Moreover, PNPX significantly inhibited or delayed the expression of chondrogenic markers including aggrecan, SOX-9 & type II collagen gene and/or protein expression. Furthermore, IHC analyses showed that a decreased expression of native CS sulphation epitopes in chondrogenic media + PNPX, suggesting the importance of their role in allowing the chondrogenic differentiation to occur.
Discussion: These results suggest that CS sulphation motifs play an important role in the differentiation of BMSCs into chondrocytes. The precise mechanism is not known, but CS sulphation motifs may be involved in the growth factor presentation needed for cell differentiation that leads to cell aggregation and extracellular matrix-cell interactions during chondrogenesis.
Referances: Hayes et al (2007) J Histochem Cytochem. 56: 125-138
Hayes et al (2011). Eur Cell Mater. 7:1-14.
Dowthwaite et al (2005) J Cell Sci. 117: 889-897

P144
A RADIOSTEREOMETRIC SPECIALISED DIRECT DIGITAL X-RAY SYSTEM: IN VITRO VALIDATION AND GEOMETRICAL ASSESSMENT
MR Downing*, GP Ashcroft
School of Medicine & Dentistry, University of Aberdeen, UK
The Adora RSA (NRT, Denmark) is a new stereo X-ray system custom built for Radeostereometry. Images are acquired using CXDI50C digital detectors (Canon, Netherlands). Analysis software was written locally to detect both Tantalum markers and the spherical head of the hip implant, and for RSA reconstruction and kinematic analysis.
To assess geometric reproducibility, a planar grid phantom was constructed with 1400 2mm markers in a grid pattern over a 350 by 430 mm glass plate. Additionally 25 tantalum markers of each diameter 1.0, 0.8 and 0.5 mm were added within a 120mm square of the grid. The phantom was imaged repeatedly with translation and rotation over the detector. For small phantom movements of up to 10mm over the detector, very small measurement errors were observed of median 2 microns, maximum 6 microns. For larger movements, the errors increased to median 5 microns and maximum 50 microns. Errors also increased with decreasing exposure.
For RSA validation, an acetabular PE cup was cemented to a Sawbone pelvis. Tantalum markers were inserted into the pelvis (10), cement (4), and cup (10). A 28mm metal head was fixed to the cup. The phantom was imaged repeatedly without movement, then moved in translation (up to 100 mm) and rotation (all axes, up to 45 degrees), and with full X-ray repositioning. Precision errors were calculated on the assumption of no relative movement between components.
Results are given for repositioning movement categorised as none, small (less than 25mm or 15 degrees), medium (less than 50mm or 30 degrees), and large. For the head, the mean total point motion error was 4, 10, 14 and 24 micrometers. Mean error of segment fitting was less than 60 microns with no markers rejected from the composite segment of 24 markers. Cup migration total translation error was 10, 16, 24, and 35 micrometers with rotation errors less than 0.05 degrees.
Observed RSA errors were small, increasing with phantom movement. This is consistent with the geometric uniformity tests. X-ray exposure and tissue thickness were also identified as factors in precision. We conclude this system has excellent precision for Radiostereometry.

P145
BACK PAIN AND OSTEOPOROSIS
VV Povoroznyuk*, TV Orlyk
Institute of Gerontology AMS, Ukraine
Research was aimed at studying the peculiarities of vertebral pain syndrome and its influence on life quality in women with postmenopausal osteoporosis in relation to localization and vertebral bodies deformation types.
Object. 353 women in postmenopausal period aged from 50 to 89 years were examined and divided into groups depending on localization and type of vertebral deformations. Research does not include the women with duration of postmenopausal period less then six months.
Methods. The questionnaire, X-ray of pectoral and lumbar spine in two projections, morphometry of vertebral analysis were used.
Results. Intensity of vertebral pain syndrome in women with osteoporosis and its complications depends on localization of deformed vertebrae. In pectoral spine intensity of pain syndrome (VAS) was higher in women with fractures of pectoral vertebrae (3,9±0,6 points, $p<0,05$) and vertebral fractures (pectoral and lumbar spine) of combined localization (3,7±1,1 points, $p<0,05$) compared with fractures present only in lumbar spine (2,7±0,7 points). In lumbar spine intensity of pain syndrome (VAS) was higher in women with fractures of combined localization (6,5±0,4 points, $p<0,05$) compared with fractures of vertebral bodies only in pectoral (4,8±0,6 points) or only in lumbar spine (5,1±0,6 points). Reliable aggravation of syndrome was explained by the presence of compression vertebral fractures in pectoral area ($p<0,05$), while in the lumbar spine there were no reliable distinctions related to the fracture occurrence.
Conclusion. The vertebral pain syndrome is observed with all types of vertebral deformations; however, its intensity is most clearly expressed in patients with compression fractures.

P146
BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROTIC FRACTURES
VV Povoroznyuk*, VM Vayda, NI Dzerovych
Institute of Gerontology AMS Ukraine
This research is aimed at studying the bone mineral density among postmenopausal women with osteoporotic fractures.
Object. The total of 160 postmenopausal women 45-79 years old (average age - 63,4±0,7 years; average duration of postmenopausal period - 14,4±0,7 years) were examined. Patients were divided into two groups: group A - women (n=100, average age - 63,2±0,9 years) without osteoporotic fractures, group B - women (n=60, average age - 65,5±1,2 years) with osteoporotic fractures in their anamnesis.
Methods. The questionnaire; measurement of anthropometrical characteristics (height, mass, body mass index); bone mineral density (BMD), T- and Z-scores of the spine (L1-L4), hip (femoral neck, trochanter and total femur), and forearm (ultradistal, midforearm) were determined by means of Dual-energy X-ray absorptiometer 'Prodigy' (GE Medical systems, 2005).

Results. All indexes of different skeletal areas measured by DXA in postmenopausal women with osteoporotic fractures were significantly lower (p<0,001) compared with the data of women without osteoporotic fractures: total body - BMD: 0,999±0,015 g/cm2 and 1,097±0,010 g/cm2, T-score: -1,59±0,18 and -0,34±0,12, Z-score: -0,81±0,15 and -0,06±0,09; spine (L1-L4) - BMD: 0,909±0,023 g/cm2 and 1,094±0,017 g/cm2, T-score: -2,26±0,20 and -0,78±0,14, Z-score: -1,18±0,18 and -0,02±0,13; femoral neck BMD: 0,780±0,016 g/cm2 and 0,886±0,014 g/cm2, T-score: -1,88±0,11 and -1,09±0,01, Z-score: -0,59±0,10 and -0,05±0,09; trochanter BMD: 0,696±0,017 g/cm2 and 0,819±0,016 g/cm2, T-score: -1,35±0,15 and -0,36±0,12, Z-score: -0,42±0,14 and 0,33±0,11; total femur BMD: 0,839±0,019 g/cm2 and 0,968±0,016 g/cm2, T-score: -1,29±0,16 and -0,27±0,12, Z-score: -0,33±0,13 and 0,45±0,11; ultradistal forearm BMD: 0,299±0,008 g/cm2 and 0,352±0,08 g/cm2, T-score: -2,12±0,20 and -0,77±0,19, Z-score: -0,74±0,21 and 0,39±0,18; midforearm BMD: 0,562±0,013 g/cm2 and 0,648±0,010 g/cm2, T-score: -2,13±0,18 and -0,96±0,12, Z-score: -0,69±0,16 and 0,18±0,12, accordingly.
Conclusion. Low bone mineral density of different skeletal areas is a significant predictor of osteoporotic fractures in postmenopausal women.

P147
EFFECT OF BONE QUALITY ON STRAIN RATE AND FRACTURE RISK
*RJ Wallace, AHRW Simpson
Orthopaedic Engineering Collaboration, University of Edinburgh, UK
There is an established link between bone quality and fracture risk. It has been suggested that reduced bone quality will also reduce the toughening mechanisms displayed during loading at a high strain rate. We hypothesised that partially decalcified bone will not demonstrate an increase in force required to cause failure when comparing low and high strain rate loading.
Mechanical properties were defined by the maximum force at failure. Bone quality was defined by the mineral content. This was altered by subjecting the bones to ultrasonically assisted decalcification in 10M EDTA to achieve an average 18% mineral reduction (A 70 yr old woman has
approx 18% of her peak bone mass). 20 pairs of sheep femurs were harvested and split into four equal groups: normal bone quality, fast strain rate (NF); normal bone quality, slow strain rate (NS); low bone quality, fast strain rate (LF) and low bone quality, slow strain rate (LS). All mechanical testing was carried out by means of 3-point bending. Load representing the slow strain rate was applied by a mechanical testing machine (Zwick) at a rate resulting in a deflection of 1mm/s. The dynamic loading was applied by a custom designed pneumatic ram at a mean rate of deflection between the specimens of 2983 mm/s (±SD 1155), this equates to strain rates experienced in a road traffic accident.
The following results for force at failure were found (mean ± SD). NF: Force 5503N (± 1012); NS: Force 3969N (± 572); LF: Force 3485N (± 772); LS: Force 3165N (± 605). Groups were compared using a Mann-Whitney U test. Significant results were found between the following groups: Normal bone quality, strain rate compared (NF-NS) p<0.002; Fast strain rate, bone quality compared (NF-LF) p=0.008; Slow strain rate, bone quality compared (NS-LS) p=0.02. No statistical significance was found when comparing low bone quality, strain rate compared (LF-LS) p=0.47.
These results show that normal healthy bone has an ability to withstand higher strain rates which protects it against fracture. This ability to withstand high strain rates is lost in decalcified bone making it more susceptible to fracture. The results of this study indicate the importance of strain rate reduction as well as energy absorption in the design of hip protectors and in environmental modifications.

P148
WHAT INFLUENCE DO INFLAMMATORY CYTOKINES HAVE ON THE DEVELOPMENT OF BIOENGINEERING STRATEGIES TO REPAIR ARTHRITIC JOINTS?
EL Williams*[1,2], C Cooper[3], ROC Oreffo[2], CJ Edwards[1]
[1]Rheumatology Department, Southampton University Hospitals NHS Trust, Southampton, UK; [2]Bone and Joint Research Group, University of Southampton Medical School, Southampton, UK; [3]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK
Despite the development of skeletal or mesenchymal stem cell (MSC) constructs aimed at creating viable cartilage and bone, few studies have examined the effects of cytokines present in rheumatoid arthritis (RA) and osteoarthritis (OA) synovial tissues, or inhibition of these, on such constructs. This work addresses these issues using both in vitro and in vivo approaches and examines potential ways of overcoming the effects of cytokines on the integrity of cartilage and bone constructs.
Synovial samples were obtained from RA or OA (n=10) patients undergoing elective hip or knee arthroplasty at Southampton General Hospital. Full ethical approval was obtained. Control bone marrow-derived stromal cells were obtained from patients undergoing emergency fractured neck of femur repair, cultured in basal, osteogenic (ascorbate and dexamethasone) and chondrogenic (transforming growth factor beta (TGFbeta3)) conditions. Differentiation towards bone and cartilage was assessed using alkaline phosphatase (ALP) staining, ALP and DNA biochemical assays and analysis of osteogenic/chondrogenic gene expression using real time polymerase chain reaction (rt-PCR). Exogenous interleukin-1 (IL-1) (10ng/mL), tumour necrosis factor alpha (TNFalpha) (10ng/mL) or interleukin-6 (IL-6) (100ng/mL) was added and effects on differentiation noted. RA and OA synovial samples were digested, cultured for 48 hours then centrifuged to produce supernatants. Cytokine profiles were determined using ELISA. These supernatants were then added to MSCs and their effects on differentiation assessed.
Mesenchymal cultures in osteogenic media with IL-1 showed an additive osteogenic effect on biochemical assays. TNF exerted a less marked and IL-6 no apparent effect on osteogenic differentiation. ALP expression by rt-PCR correlated with these findings. Addition of supernatants to mesenchymal cultures produced a marked osteogenic profile that was IL-1 and TNFalpha concentration dependent, correlating with lower supernatant dilutions on initial ELISA analysis.
Preliminary studies indicate that exogenous IL-1 and TNFalpha modulate the osteogenic phenotype in MSCs in vitro. OA and RA synovial supernatants affect skeletal cell differentiation. Variations in cytokine profiles between supernatants require analysis for potential confounders. A larger study is underway to investigate these effects, the effects of cytokines on skeletal cell differentiation on commercially available scaffolds both in vitro and in an in vivo murine model of bone formation.

P149
EFFECT OF ZOLENDRONIC ACID IN TREATMENT OF POSTMENOPAUSAL WOMEN WITH OSTEOPOROTIC VERTEBRAL FRACTURES
VV Povoroznyuk*, NV Grygorieva, VM Vaуda,NI Dzerovych, NI Balatska
Institute of Gerontology AMS Ukraine
Introduction. Zolendronic acid is a new bisphosphonate used for treatment of postmenopausal osteoporosis. We have based our findings on results of intravenous infusions of zoledronic acid in 162 cases, 25 of which - secondary.
Aim. To determine the efficacy and safety of intravenous infusions of zoledronic acid, and effects on vertebral pain, bone mineral density (BMD) in postmenopausal women with osteoporosis.
Object. 41 postmenopausal women with osteoporosis aged 49-83 years were examined: average age - 65.90±0.76 years, average height - 159.23±0.67cm, mean body mass - 67.84±1.25 kg. 50% of patients had osteoporotic vertebral fractures.
Methods. Evaluation of pain syndrome and life quality was made with questionnaires. BMD was determined with Dual-energy X-ray absorptiometer 'Prodigy' (GE Medical systems). 5 mg of zoledronic acid (Aclasta, Novartis) was administrated by intravenous injection. During the complex treatment patients received 1 tablet of calcium combined medicine (Calcium - 500 mg, Vit. D - 400 IU) 2 times a day during 12 months. Examination was performed before and after three, six, nine and twelve months of treatment course.
Results. A reliable decrease of vertebral pain syndrome by visual analogue scale was observed up to nine months. The pain syndrome increased up to twelve months. However, the given index was lower than before treatment (insignificant changes). According to EuroQol 5D scale, life quality significantly improved. BMD of spine significantly increased in comparison with indexes before treatment after three (t=5.68; p<0.00), six (t=4.88; p<0,00), nine (t=7.59; p<0,00) and twelve (t=5.55; p<0,00) months. The BMD of femur (total) increased significantly after three (t=4.76; p<0.00), six (t=8.06; p<0.00), nine (t=2.36; p=0.03) and twelve (t=2.60; p=0.02) months. Dynamics of BMD were 6.48%, 8.57% on lumbar spine and 2.75%, 3.15% on femur (total) at six and twelve months, accordingly. The BMD of forearm increased considerably after three (t=4.70; p<0.00) and twelve (t=2.30; p=0.004) months. BMD of total body significantly increased after three (t=2.65; p=0.01), six (t=3.31; p=0,003), nine (t=5.53; p<0,00) and twelve (t=2.83; p=0.01) months.
Conclusion. Intravenous infusions of zoledronic acid (5 mg) were shown to be effectively increasing BMD, decreasing pronounced vertebral pain syndrome and improving life quality in postmenopausal women with osteoporosis.

P150
HYPERLAXITY, TISSUE STRENGTH, COLLAGEN V AND SMALL LEUCINE RICH PROTEOGLYCANS EXPRESSION: IS THERE A LINK?
MA Akhtar*, CM Robinson, JF Keating, TG Ingman, D Salter, AY Muir, H Simpson<>University of Edinburgh, UK
Background:
Hyperlaxity is associated with a high incidence of sporting injuries. Collagen V regulates the diameter of fibrils of the abundant collagen type I. Decorin and biglycan are members of the small leucine rich proteoglycans(SLRP's)family and play important roles in the regulation of collagen fibrillogenesis. The aim of this study was to identify if there was a link in hyperlaxity,tissue strength,collagen V and SLRP's expression.
Patients and methods:
Data was collected for 25 patients. 12 had open shoulder stabilization and 13 had primary ACL reconstruction. Beighton score was used to assess hyperlaxity. Localization of Collagen V and SLRP's was studied by immunohistochemical staining of the paraffin embedded sections of the skin. Grading of the stain was done on a 0-4 scale(0=no staining and 4=strong

staining>50% of the slide)by three observers. Tissue specimens were mounted on a material testing system and vertical load was applied to reach yield.
Results:
The mean force required for yield in 43 tissue specimens was 70N(12-171). Data was analysed for Group A(weak group)with yield<70N(21 tissue specimens)and Group B(strong group)with yield>70N(22 specimens). The mean age was 27 years. The mean force for group A was 41N(12-67)and group B was 98N(70-171). The mean Beighton score for group A was 3.4(0-9)and Group B was 1.9(0-5). 9 specimens in Group A and 4 in Group B had Beighton score>4 indicating hyperlaxity. The mean grading of collagen V expression in skin dermal papilla was 2.4, appendages 2.2 and extracellular matrix(ECM)1.8 in group A and 1.3,1.8 and 1.7 respectively in Group B. The mean grading of decorin expression in skin was 3.2 in group A and 3.1 in Group B. The mean grading of biglycan expression in skin epidermis was 1.5,appendages 2.2,ECM in superficial dermis 1.5 and deep dermis 0.75 in group A and 1.75,2.1,1.5 and 0.5 respectively in Group B.
Conclusion:
We found that weaker tissue specimen had high incidence of hyperlaxity and increased grading of expression for Collagen V in the skin dermal papillae. No difference was found in SLRP's expression in skin in both groups. The study shows a link between hyperlaxity,tissue strength and Collagen V expression in skin dermal papillae.

P151
DOES THE ALIGNMENT OF THE FEMORAL STEM AFFECT PATIENT FUNCTIONAL OTUCOME FOLLOWING PRIMARY TOTAL HIP REPLACEMENT?
RJ Holleyman*[1], PD Gikas[1], P Tyler[1], P Coward[1], R Carrington[1], J Skinner[1], TWR Briggs[1], J Miles[1]
[1]Joint Reconstruction Unit, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex, HA7 4LP, UK
It is known that excessive varus alignment of the femoral stem in total hip replacement (THR) creates a sub-optimal biomechanical environment which is associated with increased rates of revision surgery and component wear. Little is known regarding the effect of femoral stem alignment on patient functional outcome.
Methods: Retrospective study of primary THR patients at the RNOH. Alignment of the femoral stem component in-situ was measured subjectively by a consultant musculoskeletal radiologist in both coronal and sagittal planes using post-operative anterior-posterior and lateral pelvic radiographs. Each THR was grouped into valgus, minor-valgus, neutral, minor-varus or varus coronal plane alignment and posterior, minor-posterior, neutral, minor-anterior or anterior sagittal plane alignment. Patient reported functional outcome was assessed by Oxford Hip Score (OHS) and WOMAC questionnaires. Data analysed using a linear regression model.
Results: 90 THRs were studied in 87 patients (55 Female). Mean age at THR=62 (22-86). Mean follow-up=17 months (11-39 months). Median OHS=16, WOMAC=8. Coronal plane alignment of the femoral stem was not associated with any change in OHS ($p>0.05$) or WOMAC score ($p>0.05$). Sagittal plane alignment of the femoral stem was not associated with any change in OHS ($p>0.05$) or WOMAC score ($p>0.05$).
Conclusion: Although it is known that alignment of the femoral stem on sagittal and coronal planes has a direct effect on survivorship of the prosthesis, our study does not demonstrate any relationship between femoral stem alignment and functional outcome in patients undergoing primary THR.

P152
HYPERLAXITY, CAPSULE STRENGTH, COLLAGEN V AND SMALL LEUCINE RICH PROTEOGLYCANS EXPRESSION: IS THERE A LINK?
MA Akhtar*, CM Robinson, JF Keating, TG Ingman, D Salter, AY Muir, H Simpson
University of Edinburgh, UK
Background:
Hyperlaxity is associated with a high incidence of shoulder dislocations. Collagen V regulates the diameter of fibrils of the abundant collagen type I. Decorin and biglycan are members of the small leucine rich proteoglycans(SLRP's)family and play important roles in the regulation of collagen fibrillogenesis. The aim of this study was to identify if there was a link in hyperlaxity,capsule strength,collagen V and SLRP's expression.
Methods:
Data was collected for 10 patients undergoing open shoulder stabilization for recurrent instability. Beighton score was used to assess hyperlaxity. Localization of Collagen V and SLRP's was studied by immunohistochemical staining of paraffin embedded sections of shoulder capsule. Grading of the stain was done on a 0-4 scale(0=no staining and 4=strong staining>50% of the slide)by three observers. Shoulder capsules were mounted on a material testing system and vertical load was applied to reach yield.
Results:
The mean force required for yield in 15 shoulder capsules was 45N(17-78). Data was analysed for Group A(weak group) with yield<45N(8 specimens) and Group B(strong group)with yield>45N(7 specimens). The mean age was 26 years and all were male. The mean force for group A was 31N(17-41) and group B was 59N(45-78). The mean Beighton score for group A was 1.9(0-4) and Group B was 2. 2 specimens in Group A had Beighton score>4 as compared to 0 in Group B, indicating hyperlaxity. The mean grading of collagen V expression in synovial surface was 2.6,Blood vessels(BV)1.6 and extracellular matrix(ECM)1.9 in Group A and 4,3.1 and 2.6 respectively in group B. The mean grading of decorin expression for shoulder capsule was 2.7 in Group A and 3.3 in group B. The mean grading of Biglycan expression in synovial surface was 2,BV 2 and ECM 2.9 in Group A and 2,2.5 and 4 respectively in group B.
Conclusions:
We found that weaker capsule specimen(group A)had higher incidence of hyperlaxity. Decorin and biglycan expression in ECM and Collagen V expression in synovial surface,BV and ECM of shoulder capsule was higher in group B(strong group). This study shows a link between hyperlaxity,strength,Collagen V and SLRP's expression in shoulder capsule.

P153
IS THE WII FIT FOR PURPOSE? VALIDATING NINTENDO'S CHOSEN MEASURE OF BALANCE FOR CLINICAL USE
JJ Negus*[1,2], BA Mani[1,2], CJ Scholes[1], DA Parker[1]
[1]Sydney Orthopaedic Research Institute, Australia; [2]University of Sydney, Australia
Medical and allied health staff are beginning to incorporate the Nintendo Wii-Fit into musculoskeletal rehabilitation protocols. One potential application is the assessment of standing balance following Orthopaedic lower limb surgery. The Wii Balance Board (WBB) has been shown to be a valid equivalent to a laboratory grade force platform for the assessment of standing balance. Our objective was to investigate the validity and reliability of the balance tests included with the Wii-Fit software.
Initially, a single subject performed multiple repeats of a standing balance test. The data was collected simultaneously from a commercial force platform using its integrated software that measured centre of pressure and from the WBB using the Wii-Fit software that generated a percentage score. The data from each was compared and analyzed, applying the equations of known, validated standing balance measurements.
Then, thirty subjects free of lower limb pathology performed a series of standing balance tests combining single leg and double leg stance with their eyes open and then closed. Data was collected from one set of trials on the WBB using the Wii-Fit software and another using bespoke centre of pressure software on a laptop computer. The tests were then repeated on a second occasion within 2 weeks.
The algorithm used by the Wii-Fit software to generate the 'Stillness' standing balance score was calculated with a predictive value (R squared) of 0.94. This correlated well to a known, valid measure of standing balance.
Test-retest reliability was examined for the data from both pieces of software. Both demonstrated good-to-excellent test-retest reliability within 'software'. The laptop data was transformed using the algorithm and the between 'software' reliability was calculated as good-to-excellent.
The Wii-Fit software collects standing balance data from the WBB at a fraction of the cost of laboratory grade systems. The score generated by the Wii-Fit software is reliable and valid as an overall assessment of standing balance. Although its application would be limited for detailed assessment of balance disorders, it could still provide surgeons with an affordable, clinic based balance-screening tool. This could form part of an assessment protocol following lower limb surgery.

P154
AN IN VITRO STUDY INTO THE EFFECT OF ZINC-SUBSTITUTED HYDROXYAPATITE ON OSTEOCLAST NUMBER AND ACTIVITY
D Shepherd*[1], K Kauppinen[2], N Rushton[3], S Best[1], R Brooks[3]
[1]Department of Materials Science and Metallurgy, University of Cambridge, UK; [2]Department of Anatomy and Cell Biology, University of Oulu, Finland; [3]Orthopaedic Research Unit, University of Cambridge, UK
The aseptic loss of bone after hip replacement is a serious problem leading to implant instability. Hydroxyapatite coating of joint replacement components produces a bond with bone and helps to reduce loosening. However, over time bone remodeling at the implant interface leads to loss of hydroxyapatite. One possible solution would be to develop a coating that reduces hydroxyapatite and bone loss. Hydroxyapatite can be chemically modified through the substitution of ions to alter the biological response. Zinc is an essential trace element that has been found to inhibit osteoclast-like cell formation and decrease bone resorption. It was hoped that by substituting zinc into the hydroxyapatite lattice, the resultant zinc-substituted hydroxyapatite (ZnHA) would inhibit ceramic resorption and the resorption of bone. The aim of this work was to investigate the effect of ZnHA on the number and activity of osteoclasts.
Discs of phase pure hydroxyapatite (PPHA), 0.37wt% ZnHA and 0.58wt% ZnHA were produced, sintered at 1100 degrees Celsius and ground with 1200 grit silicon carbide paper. They were cultured in medium containing macrophage colony stimulating factor and receptor activator of nuclear factor kappa B ligand (RANKL) for 11 and 21 days. A control disc of PPHA cultured in medium containing no RANKL was also used. On the required dates the discs were removed and the cells stained for actin with phalloidin-TRITC and

the cell nuclei with 4',6-Diamidino-2-phenylindole dihydrochloride. Cells with 3 or more nuclei were classed as osteoclasts and counted using ImageJ. On day 21 after the cells had been counted, the cells were removed and the discs coated in platinum before viewing with a scanning electron microscope. Resorption areas were then measured using ImageJ.
The addition of zinc was observed to significantly decrease the number of differentiated osteoclasts after 21 days ($p<0.005$ for 0.58wt% ZnHA compared to PPHA and $p<0.01$ for 0.37wt% ZnHA compared to PPHA). The area of resorption was also significantly decreased with the addition of zinc ($p<0.005$ for the comparison of 0.58wt% ZnHA with PPHA)
The work found that zinc substituted hydroxyapatite reduced the number and subsequent activity of osteoclasts.

P155
THE EFFECT OF MOBILITY AID ON THE TOTAL HEART BEAT INDEX OF PARALYMPICS ATHLETES WHEN TRAVERSING COMPLEX TERRAINS
N Papadosifos*[1], D Boampong[1], R Weiler[1], P Smitham[1], N Tyler[1], C Holloway[1], S Grange[2]
[1]University College London, UK; [2]University of Calgary, Canada
Introduction: In the US over half a million people are prescribed crutches each year. More than 750,000 wheelchair users exist in the UK and wheelchair and crutch users commonly develop shoulder pathology. The purpose of this study was to determine the influence of complex topographies on heart rate (HR) and thus energy expenditure, using a wheelchair and differing crutch designs on the exertional body stress.
Method: Two Paralympics Athletes from the GB amputee football squad were assessed in a Lomax Active wheelchair and 5 different types of crutches in a randomly allocated order over a course representing everyday complex terrains at the Pedestrian Accessibility and Movement Environment Laboratory (PAMELA), University College London. In addition results were compared over the same course with the athletes using their own personal pair of crutches. The PAMELA course consisted of a mixture of 4% and 2.5% cross falls (transverse) and a simulated road crossing, sprint, slalom and a slow straight.
Results: Initial findings show both athletes needed to work harder, thus spend more energy (13% more) to cope with the wheelchair tasks (2.6) than with the crutches(2.3). The Total Heart Beat Index (THBI) revealed that trying to ambulate with the crutches was more difficult in 4% cross fall (3.3) than on the longitudinal slopes (3.2), followed by 2.5% cross fall (2.85), slalom (2.1) and sprint (1.8). For the same tasks executed using a wheelchair the 2.5% gradient was shown to be the higher energy demanding (3.8), followed by the 4% (3.5), slopes (2.9), slalom (2.2) and sprint (2.1). Both participants reached a lower THBI (2.2) during the same task when using their own crutches.
Conclusion: The results of this study imply that ambulation with crutches puts less burden than wheelchair. This might be due to the time these athletes spend with crutches, either in training or activities of daily living. Furthermore, the physical strain which they underwent during the complex terrains was clearly reflected on their heart rate. The setting of longer distances to collect more consistent HR data should be the focus of further research. The comparison in performance between athletes and the general population should also be investigated.
Keywords: Paralympics, Total Heart Beat Index (THBI), Lomax Active wheelchair, crutches, amputee

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CAN THE ROTATIONAL SPEED TO CAUSE SPIRAL FRACTURE BE ESTIMATED FROM THE FRACTURE PATTERN?
PS Theobald[1], A Qureshi*[2], MD Jones[1]
[1]Trauma Biomechanics Research Group, School of Engineering, Cardiff University, CF24 3AA, UK; [2]Division of Orthopaedic Surgery, Leicester General Hospital, Gwendolen Road, Leicester, LE5 4PW, UK
Long bone fractures are a commonly presented paediatric injury. Whilst the possibility of either accidental or non-accidental aetiology ensures significant forensic relevance, there remain few clinical approaches that assist with this differential diagnosis. The aim of this current study was to generate a reproducible model of spiral fracture in immature bone, allowing investigation of the potential relationship between the rotational speed and the angle of the subsequent spiral fracture.
Seventy bovine metacarpal bones were harvested from 7 day old calves. Sharp dissection ensured removal of the soft tissue, whilst preserving the periosteum. The bones were then distributed evenly before eleven groups, before being aligned along their central axis within a torsional testing machine. Each group of bones were then tested to failure at a different rotational speed (0.5, 1, 15, 20, 30, 40, 45, 60, 75, 80 and 90 degrees s-1). The angle of spiral fracture, relative to the long axis, was then measured, whilst the fracture location, the extent of comminution and periosteal disruption, were all recorded.
Sixty-two out of 70 specimens failed in spiral fracture, with the remaining tests failing at the anchorage site. All bone fractures centred on the narrowest waist diameter, with 5 specimens (all tested at 90 degrees s-1) demonstrating comminution and periosteal disruption. The recorded spiral fracture angles ranged from 30 - 45 degrees, and were dependant on the rotational speed.
This study has established a relationship between the speed of rotation and the angle of spiral fracture in immature bovine bone. It is anticipated that further study will enable investigation of this trend in paediatric bone, ultimately providing an additional diagnostic tool for clinicians trying to verify the proposed mechanism of injury.

P157
RETRIEVAL ANALYSIS OF TISSUE ENGINEERED BONE: A CLINICAL AND LABORATORY STUDY
A Aarvold*[1,2], JO Smith[1], ER Tayton[1], AMH Jones[2], JI Dawson[1], A Briscoe[1], S Lanham[1], DG Dunlop[2], ROC Oreffo[1]
[1]Bone and Joint Research Group, University of Southampton, UK; [2]Southampton General Hospital, UK
Background: Skeletal stem cells can be combined with human allograft, and impacted to produce a mechanically stable living bone composite. This strategy has been used for the treatment of femoral head avascular necrosis, and has been translated to four patients, of which three remain asymptomatic at up to three year follow-up. In one patient collapse occurred in both hips due to widely distributed and advanced AVN disease, necessitating bilateral hip arthroplasty. However this has provided the opportunity to retrieve the femoral heads and analyse human tissue engineered bone.
Aims: Analysis of retrieved human tissue-engineered bone in conjunction with clinical follow-up of this translational case series.
Methods: A parallel in vitro culture of the implanted cell-graft constructs was set up at the time of surgery, with serial cell viability stains performed up to six weeks. Patient follow-up was by serial clinical and radiological examination. Tissue engineered bone from the two retrieved femoral heads was analysed histologically by Alcian blue & Sirius red stain and bi-refringence, by micro computed tomography (microCT) for both bone density and morphology, and by compression testing for mechanical strength. Normal trabecular and cortical bone from the femoral heads was used as controls.
Results: Parallel in vitro analysis demonstrated sustained cell growth and viability on the allograft. Histologically, the retrieved tissue engineered specimens demonstrated a mature trabecular micro-architecture and organization identical to normal trabecular bone. MicroCT revealed trabecular morphology within the tissue-engineered bone, with bone density of 1400 Grey scale units (compared to 1200 for natural trabecular bone and 1800 for cortical bone). Axial compression testing showed no difference in strength between engineered and trabecular bone.
Conclusions: Widespread residual necrosis in the femoral heads of one patient resulted in collapse requiring hip arthroplasty, but analysis of the tissue engineered bone sections has demonstrated the translational potential of a living bone composite to restore both the biological and mechanical characteristics of bone defects. Clinical follow-up shows this to be an effective new treatment for focal early stage avascular necrosis of the femoral head, and this unique retrieval analysis data confirms the potential of cell-based strategies for clinical treatment of bone defects.

P158
OUTCOME OF THRUST PLATE PROSTHESIS AFTER A MINIMUM OF FIVE YEARS
KH Sunil Kumar*[1], SC Budithi[1], A Jaiswal[1], E Robinson[2], JB Richardson[2]
[1]Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry, UK; [2]Institute of Orthopaedics, Oswestry, UK
Background: Thrust plate prosthesis (TPP) is a bone conserving prosthesis in use for over thirty years. TPP is a stemless and uncemented femoral prosthesis fixed at the lateral femoral cortex with a bolt, plate and screw. This has a metal-on-metal articulation with a 28mm Metasul head and Allofit press fit acetabular cup. Our study aimed to assess the functional outcome of this prosthesis.
Methods: In our institution 234 TTPs were implanted between 1995 and 2005. All patients completed a self-assessed questionnaire of Harris Hip Score at 2 months, 1 year, and then yearly. Only those who had a follow up was within the last two years were included in the analysis. 76 patients who had failed to satisfy the criteria were excluded. Of the 158 hips in the study 75 hips were in male patients and 83 were in female patients. The median age of patients was 52 years (range 15 to 82). 75 hips were on the right side and 83 on the left. All patients were operated by the senior author or a senior trainee under his supervision (seven hips). Revision of the implant or decision to revise was taken as the end point of our study.
Results: The median time to follow up was 7 years (range 1 to 15). The median pre-operative hip score was 43 (range 3 to 77) which rose to 83 points (range 11 to 100) at the latest follow up. Median hip score in females improved from 39 to 82 points and in males from 52 to 85 points. Twelve patients underwent revision surgery either for infection or aseptic loosening.
Conclusion: The Thrust Plate Prosthesis had a good outcome with an increase in hip score of 40 points and a median survival of 7 years.

P159
FUNCTION AFTER REVISION OF ACETABULUM IN HIP RESURFACING
KH Sunil Kumar*[1], A Jaiswal[1], R Gilbert[1], A Carrothers[1] JH Kuiper[2], JB Richardson[2]

[1]Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry, UK; [2]Keele University, UK

Background: Hip resurfacing has resurged in the last decade due to a renewed interest in metal on metal bearing. One of the proposed advantages is ease of revision of the femoral component. Short term functional results after femoral revision are similar to those after conventional total hip replacement. Survival and function after revision of the acetabular component only or of both components have not been reported. We aimed to assess hip function and implant survival after revision of the acetabular component for failed Birmingham hip resurfacing (BHR).

Methods: The Oswestry Outcome Centre collected data prospectively on 5000 patients who underwent hip resurfacing between 1997 and 2002. Of these, 182 hips were revised: 42% had revision of the femoral component only, 8% revision of the acetabular component only, and 50% revision of both components. This study analyzed patients who had revision of the acetabular component, either in isolation or in combination with the femoral component.

Results: In the isolated acetabular revision group the median Harris Hip Score was 74 at a mean of 4.5 years post-revision. In the both components revision group the median Harris hip score was 85 at a mean of 4 years. There was no significant difference in function between the groups. Kaplan-Meier survivor ship analysis after revision showed an average survival of 91% at 10 years. There was a significant difference between survival of isolated acetabular revision (75%) and both component revision (96%).

Conclusions: Revision total hip replacement subsequent to failure of hip resurfacing has good outcome and good midterm survival. Isolated acetabular revision and revision of both components had similar function but survival was significantly worse in the isolated acetabular revision group.

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THE EFFECT OF HIP JOINT CENTRE OF ROTATION DISPLACEMENT ON PATIENT FUNCTIONAL OUTCOME FOLLOWING PRIMARY TOTAL HIP REPLACEMENT

RJ Holleyman*[1], PD Gikas[1], P Tyler[1], P Coward[1], R Carrington[1], J Skinner[1], TWR Briggs[1], J Miles[1]

[1]Joint Reconstruction Unit, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex, HA7 4LP, UK

Background: The position of the hip-joint centre of rotation (HJC) within the pelvis is known to influence functional outcome of total hip replacement (THR). Superior, lateral and posterior relocations of the HJC from anatomical position have been shown to be associated with greater joint reaction forces and a higher incidence of aseptic loosening. In biomechanical models, the maximum force, moment-generating capacity and the range of motion of the major hip muscle groups have been shown to be sensitive to HJC displacement. This clinical study investigated the effect of HJC displacement and acetabular cup inclination angle on functional performance in patients undergoing primary THR.

Methods: Retrospective study of primary THR patients at the RNOH. HJC displacement from anatomical position in horizontal and vertical planes was measured relative to radiological landmarks using post-operative, calibrated, anterior-posterior pelvic radiographs. Acetabular cup inclination angle was measured relative to the inter-teardrop line. Maximum range of passive hip flexion, abduction, adduction, external and internal rotation were measured in clinic. Patient reported functional outcome was assessed by Oxford Hip Score (OHS) and WOMAC questionnaires. Data analysed using a linear regression model.

Results: 109 THRs were studied in 104 patients (69 Female). Mean age at THR=63 years (22-88). Mean follow-up=17 months (11-39 months). Median OHS=16, WOMAC=8. Increasing vertical HJC displacement (in either superior or inferior direction) from anatomical position was associated with worsening OHS ($p<0.05$) and WOMAC scores ($p<0.05$) and a reduced range of passive hip flexion ($p<0.05$). No relationship was found between either horizontal HJC displacement or acetabular cup inclination angle and patient functional outcome.

Conclusion: A significant relationship was identified between increasing vertical displacement of the HJC and worsening patient functional outcome. This supports current opinion regarding the disadvantageous consequences of a superiorly displaced HJC in terms of survivorship and function. We therefore advocate an anatomical restoration of HJC position wherever possible.

P161

OUTCOME OF COMBINED AUTOLOGOUS CHONDROCYTE IMPLANTATION AND ALLOGRAFT MENISCUS TRANSPLANTATION

KH Sunil Kumar*[1], R Gilbert[1], A Bhosale[2], P Harrison[2], JB Richardson[2]

[1]Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry, UK; [2]Institute of Orthopaedics, Oswestry, UK

Background: Autologous Chondrocyte Implantation (ACI) is frequently used to treat chondral defects in the knee with a good long-term outcome. This is contraindicatd in meniscal deficient knees. Allogenic Menicsal Transplantation (AMT) has been shown to give good symptomatic relief in meniscus deficient knees. However this is contraindicated in advanced cartilage degeneration. We hypothesized that combination of these two might be a solution for bone-on-bone arthritis in young individuals.

Methods: We studied a consecutive series of 12 patients who underwent combined ACI and AMT between 1998 and 2005. Pre operative and post operative comparisons of lysholm scores were recorded. Magnetic Resonance Imaging was performed to assess the integration ACI & AMT. Arthroscopy was performed at one year for assessment and obtain biopsy for histological examination.

Results: Out of the twelve patients only eleven were included as one had died at three months after surgery. The median pre-operative lysholm score was 45 which rose to 64 at one year. Magnetic Resonance Imaging showed good integration of both ACI and menisci. Most of the patients were able to lead an active lifestyle.

Conclusion: The combination of both ACI & AMT could give a good result and defer a total knee replacement in young indi

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AUTOLOGOUS CHONDROCYTE IMPLANTATION TO TREAT CHONDRAL AND OSTEOCHONDRAL DEFECTS IN THE HIP

KH Sunil Kumar*[1], VG Murakibhavi[3], S Roberts[2], F Guerra-Pinto[4], E Robinson[2], PE Harrison[2], DC Mangam[1], I McCall[1], JB Richardson[1,2]

[1]Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry, UK; [2]Institute of Orthopaedics, Oswestry, UK; [3]JN Medical College, KLES Dr Prabhakar Kore Hospital & Medical Research Centre, Belgaum, India; [4]Serviço de Ortopedia, Hospital Curry Cabral, Lisbon, Portugal

Background: Autologous Chondrocyte Implantation (ACI) is a procedure which is gaining acceptance for the treatment of cartilage defects in the knee with good results and a long term durable outcome. Its use in other joints has been limited, mainly to the ankle. We aimed to assess the outcome of ACI in the treatment of chondral and osteochondral defects in the hip.

Methods: Fifteen patients underwent ACI for chondral or osteochondral defects in the femoral head with a follow up of upto 8 years (mean of 2 years) in our institution with a mean age of 37 years at the time of operation. Pre-operatively hip function was assessed by using the Harris Hip Score and MRI. Post-operatively these were repeated at 1 year and hip scores repeated annually. Failure was defined as a second ACI to the operated lesion or a conversion to a hip resurfacing or replacement.

Results: The mean pre-op Harris Hip Score (HHS) was 55 which increased to 63 at 1 year and 70 at the latest follow up. Patients who underwent ACI for cartilage defects secondary to trauma (four) were better with a mean HHS of 69 at a mean follow up of 3.5 years. Six patients underwent THR at a mean of 32 months and were classed as failures. Five patients had evidence of avascular necrosis (AVN) of the femoral head post operatively of which four AVN pre-op.

Conclusion: These early results suggest that ACI could be a viable option for the treatment of isolated chondral defects in the hip. The presence of AVN or bone cysts pre-op may be a predictor of failure.

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EFFECTS OF X-RAY IRRADIATION ON LOAD PARTITIONING BETWEEN THE CONSTITUENT PHASES OF CORTICAL BONE

AC Deymier-Black*[1], A Singhal[1], F Yuan[1],JD Almer[2], SR Stock[3], LC Brinson[4], DC Dunand[1]

[1]Department of Materials Science and Engineering, Northwestern University, Evanston, IL, USA; [2]Advanced Photon Source, Argonne National Laboratory, Argonne, IL, USA; [3]Department of Molecular Pharmacology and Biological Chemistry, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [4]Department of Mechanical Engineering, Northwestern University, Evanston, IL, USA

X-ray Irradiation is known to have significant effects on the fracture properties of cortical bone due to the cross-linking and degradation of collagen [1]. However, no information exists on the effect of x-ray irradiation on the partitioning of load between the stiffer hydroxyapatite (HAP) and the weaker collagen phases of bone, which is dependent on the quality of the interface between the two phases. Here, we use a combination of small and wide-angle high-energy x-ray scattering to measure the HAP and fibrillar strains (and thus loads) of bovine bone, as has been previously done on bovine dentin [2], under various compressive loading schemes and at different doses of x-ray irradiation.

As the x-ray dose increases, we find a decrease in both the HAP residual strain and the elastic strain carried at steady state during bone creep deformation under a compressive load. This load shedding by the HAP platelets suggests that damage at the HAP-collagen interface limits the efficiency of load transfer. However, the load carrying capacity of HAP during rapid load-unload experiments (unlike long-term creep experiments) shows no change with increasing irradiation levels.

This combination of HAP load bearing behaviors might be caused by reversible interfacial damage, rather than irreversible collagen cross-linking. The interfacial bond between the HAP platelets and the collagen is mainly composed of weak electrostatic bonds. Decarboxylation of the collagen side chains upon irradiation is known to lead to bond breakage [3], allowing relaxation of the HAP strain. We hypothesize that these same weak bonds are also easily reformed, allowing the interface to regain its original load transfer abilities.

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P164
Vitamin D deficiency and poor bone health are associated with CVD risk in Caucasian and Asian women
OA Hakim*[1], F Shojaee-Moradie[2], K Hart[1], JL Berry[3], R Eastell[4], F Gossiel[4], R Hannon[4], AM Umpleby[2], BA Griffin[1], SA Lanham-New[1]
[1]Faculty of Health and Medical Sciences, Postgraduate Medical School, University of Surrey, Guildford, [3]Vitamin D Research Group, University of Manchester, [4]Department of Human Metabolism, University of Sheffield, UK
Late abstract

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RISK OF AVASCULAR NECROSIS OF THE FEMORAL HEAD AFTER INTRA-ARTICULAR STEROID INJECTION
SP Mackenzie, MA Akhtar*, ND Clement, AM Kerr, CH Tiemessen, SA Mehdi
Trauma and Orthopaedic Department, Borders General Hospital, UK
Intra-articular steroid injection with local anaesthetic is commonly used as a diagnostic tool to differentiate between hip and spine pathology. The aim of our study was to identify the risk of avascular necrosis (AVN) after intra-articular steroid injection of the hip.
We conducted a retrospective review of 103 patients who underwent intra-articular steroid injection of the hip from 2007 to 2010 (without radiographic signs of AVN before the injection) in a district general hospital. The grade of the osteoarthritis was recorded pre and post injection using the Kellgren and Laurence radiological classification. A control group of 103 patients matched for age, gender, and grade of osteoarthritis was used for comparison. Patient's notes were reviewed to identify the risk factors for AVN.
The mean age was 67.9 years and 68% were female. The mean time between radiographs was 21.9 months for the injection group and 19.3 months for the control group. Eight patients (7.8%) in the injection group developed AVN, of which only one had an identifiable risk factor. The risk of developing AVN after steroid injection was significantly higher. (OR 8.6, p=0.041). The mean grade of the osteoarthritis progressed from 2.1 to 2.7 in the injection group and from 2.2 to 2.9 in the control group.
Intra-articular steroid injection of the hip carries a significant risk of AVN of the femoral head and should be used cautiously. Further studies using micro CT scan to look at the micro fractures following intraarticular steroid injections leading to AVN are needed.

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Abstract withdrawn

INVITED SPEAKER PROFILES

Nigel Arden
Nigel Arden is Professor in Rheumatic Diseases and Consultant in Rheumatology, University of Southampton Director of Biobank and Musculoskeletal Epidemiology; and Deputy Director NIHR Biomedical Research Unit, University of Oxford.

Professor Arden trained at St Thomas's Hospital, London, where he also completed four years of research into the genetics of osteoporosis. During this time, he gained an MSc in Epidemiology and an MD.

He moved to the South Coast in 1996 and in 1998 spent six months as Visiting Assistant Professor in Epidemiology at the University of San Francisco.

In 2000 he commenced his post as Senior Lecturer in Rheumatology and Honorary Consultant Rheumatologist at Southampton University NHS Trust. In 2008, he commenced an appointment with the University of Oxford to develop a joint research department between the two universities of Oxford and Southampton. He is based jointly at the Medical Research Council Epidemiology Resource Centre, Southampton and the Botnar Research Centre, Oxford; where he continues his research into osteoarthritis and osteoporosis.

He has published 107 research papers and written 5 books in the field of rheumatology. Currently Nigel is a member of the National Osteoporosis Society Scientific Advisory Board; and sits on the EULAR (European League Against Rheumatism) Osteoarthritis Guideline Committee and OARSI (Osteoarthritis Research Society International) Committees for the Treatment of Osteoarthritis and the Use of MRI in the diagnosis of Osteoarthritis.

Sir Michael J Berridge
Michael Berridge is an Emeritus Babraham Fellow at the Babraham Institute in Cambridge.
His main area of research interest concerns the role of calcium in cellular control processes with particular emphasis on neural signalling, cardiac contractility and cell proliferation. He became a Fellow of Trinity College in 1972 and was elected a Fellow of The Royal Society in 1984. For his work on second messengers Berridge has received numerous awards and prizes, including The Louis Jeantet Prize in Medicine, The Albert Lasker Medical Research Award, The Heineken Prize, The Wolf Foundation Prize in Medicine and The Shaw Prize. In 1998 he was knighted for his service to science.

Gordon Blunn
Professor Blunn has been the Head of the Centre of Bioengineering at The Institute of Orthopaedics and Muskuloskeletal Science at University College London since 2000. Professor Blunn's experience lies in materials and design of orthopaedic implants. Recent projects include the development of a non-invasive growing prosthesis used to treat bone cancers in children where the prosthesis is able to be extended in a non-invasive manner to keep growth of the affected leg in line with that of the normal limb. Other developments include the use of an Intraosseous Transcutaneous Amputation Prosthesis which has been used in amputees to securely fix the artificial limb to the skeletal bone. Projects such as these require expertise.

Roger Brooks
Dr Brooks is a Senior Research Associate in the Orthopaedic Research Unit, University of Cambridge based at Addenbrooke's Hospital. His research interests include evaluating scaffolds for musculoskeletal tissue engineering, investigating cell responses to particulate materials, inhibiting inflammatory osteolysis and in vitro and in vivo models of bone repair. He is currently funded by the National Institute for Health Research through the Cambridge Biomedical Research Centre Musculoskeletal Program and is involved in a number of projects with industrial partners centred on the repair of bone, cartilage and tendon. He is also involved in several academic collaborations including with the Cambridge Centre for Medical Materials evaluating nanocomposites, biodegradable polymers and a range of bioactive ceramics as implant materials.

Chris Burgoyne
Chris Burgoyne is a Structural Engineer and Head of the Structures Group at the University of Cambridge. He took his PhD at Imperial College where he taught before taking up his current post in 1989. He has interests in the use of new materials such as advanced fibres in structural engineering applications, but has also undertaken research into various aspects of bone mechanics, including the structural behaviour of human ribs, interpretation of fossil bones, and most recently the behaviour of the femoral neck, where it has been shown that it is buckling resistance rather than material strength that is the critical factor.

David Burr
David B Burr is Professor of Anatomy and Cell Biology and Professor of Biomedical Engineering at Indiana University. Dr Burr studies the response of bone to mechanical stimuli, pharmaceutical treatments for osteoporosis, cartilage and bone repair in arthritis, and the biomechanics of stress fractures. He is the author of more than 200 peer-reviewed articles, 23 book chapters and four books on the structure, mechanics and fracture of bone.

Dr Burr is Past-President of the American Association of Anatomists, and Past-President of the Orthopaedic Research Society. He won the Borelli Award from the American Society of Biomechanics in 2008 and the Gideon A. Rodan Excellence in Mentorship Award from the ASBMR in 2010. He is an Associate Editor for *Bone*, and the *J of Musculoskeletal and Neuronal Interactions*, and serves on the editorial boards for *J of Biomechanics, Osteoporosis International, Calcified Tissue International*, and *J of Bone and Mineral Metabolism*.

Patrick Case
Consultant Senior Lecturer in Orthopaedic Surgery and Pathology at the University of Bristol

Bruce Caterson
B.Sc. Hons. (1971) & Ph.D (1976) in Biochemistry from Monash University, Victoria, Australia. Postdoc – Professor in USA from 1975-1995 (in Alabama, West Virginia & North Carolina) and now 16 years in UK in Cardiff. Currently, Professor of Biochemistry, School of Biosciences & Associate Director of Musculoskeletal Research, School of Medicine, Cardiff University, Wales, UK.
Research Interests: Anything to do with the structure, function & metabolism of connective tissues in health & disease; especially musculoskeletal tissues and more recently stem cells & tissue regeneration/repair.
Service: 1993 - President, Orthopaedic Research Society & 1988-1996 Board of Directors, Orthopaedic Research Society (USA); 2000-2002 President, Society for Back Pain Research (UK); 2004-2007 President, British Orthopaedic Research Society; 2002-2009 Chairman, British Society for Matrix Biology.
Honours: 1987 - Benedum Distinguished Scholar Award in Bioscience and Medicine at West Virginia University (USA); 1998 - *Kappa Delta Elizabeth Winston Lanier Award for Outstanding Orthopaedic Research* from the American Academy of Orthopaedic Surgeons and Orthopaedic Research Society (USA);2009 - *Barry Preston Award* for contributions to Australian & New Zealand Matrix Biology; 2011 - *Fell-Muir Award* for outstanding contributions to British Matrix Biology.
Publications: Total 195 (168 Full Papers & 27 Reviews/Book Chapters).

Chantal Chenu
Chantal Chenu is a Senior Lecturer in Bone Cell Biology at the Royal Veterinary College in London. After graduating from the University of Lyon with a degree in Biochemistry, she conducted her PhD research in David Roodman's laboratory in San Antonio, Texas on osteoclast differentiation from bone marrow. She then joined Pierre Delmas' INSERM group in Lyon, where her work focused on the biochemical and functional characterization of several bone matrix proteins. She became an independent researcher with INSERM in 1991, investigating the role of the nervous system in the control of bone development and turnover. She was a co-founder of the French Society on Biology of Mineralised Tissues in 1997. She moved to the Royal Veterinary College in 2003, where her research has focused on the regulatory and repair mechanisms of bone. Her recent research, funded by the Wellcome Trust, is aimed at investigating the control of bone mass in relation to energy metabolism, with a particular interest on the role of the energy sensor AMP-activated protein kinase (AMPK). Chantal Chenu has served on the Editorial Board of Journal of Bone and Mineral Research and is a member of the Faculty of 1000 Medicine.

Juliet Compston
Juliet Compston is Professor of Bone Medicine and Honorary Consultant Physician at the University of Cambridge School of Clinical Medicine, a position she took up in 2003. Her research is focused on the pathophysiology of osteoporosis and the cellular and structural mechanisms by which pharmacological interventions preserve bone mass and reduce fracture risk. She has conducted studies into the pathophysiology of bone disease in a number of disorders, including postmenopausal osteoporosis, post-transplantation osteoporosis and cystic fibrosis. Recently her research has focused on fractures in obese postmenopausal women.

Professor Compston is a past President of the Bone and Tooth Society of Great Britain, as well as a past Chairman and President of the International Society of Bone Morphometry. She is currently a member of the Board of the International Osteoporosis Foundation (IOF) and its Committee of Scientific Advisors, and a Trustee of the Medical Board of the National Osteoporosis Society. She is Chair of the European Union Osteoporosis Consultation Panel and of the UK National Osteoporosis Guidelines Group.

Professor Compston is Associate Editor of the *Journal of Bone and Mineral Research* and a member of the Editorial Board of several peer-reviewed journals including *Bone*, *Osteoporosis International*, *Calcified Tissue International* and the *Journal of Clinical Densitometry*. She has published over 300 original research papers and reviews.

In 2006, Professor Compston was awarded the National Osteoporosis Society Kohn Foundation Award, and in 2009, the International Bone and Mineral Society John G Haddad Jr Award and the ASBMR Frederic C Bartter Award.

John Currey
John Currey is emeritus Professor of Biology at the University of York, UK.
He was educated at Oxford, where he had a firm grounding in evolutionary matters, which remain a great interest of his. He moved into biomechanics almost by accident (he can still remember having his hand on the door to leave when the engineer to whom he was talking said 'Why don't you test it?).
Since then he has had a constant interest in the mechanics of bone and other hard tissues like mollusc shells, first in Oxford, then till his retirement and after at York, except for a year in Cleveland Ohio, where he was taught what little formal biomechanics he has learnt by Al Burstein.

Jean-Marie Delaisse
Dr Delaisse entered the bone field in 1979, at the Institute of Cellular Pathology (Brussels) directed by C. de Duve. His research, inspired by the lysosomal concept, aimed first at identifying the proteinases solubilizing the bone matrix, and broadened later to the role of proteinases in regulation of bone remodeling before and after the main bone solubilization step. In 1995, Dr Delaisse moved to the Center of Clinical and Basic Research / Nordic Bioscience (Copenhagen) to continue this research as CSO. In 2003, he moved to the University of Southern Denmark (Odense), where he became Professor of Clinical Cell Biology, and investigates the remodeling mechanism of adult human bone, paying special attention to the supracellular organization of the remodeling area.

James Edwards
James Edwards, D. Phil., graduated from, University of Oxford before studying with Dr. Gregory Mundy at the University of Texas, and helped found the Vanderbilt Centre for Bone Biology, Nashville. He currently holds a faculty position at the Institute of Musculoskeletal Sciences, University of Oxford, studying ageing mechanisms in normal and pathological musculoskeletal tissues. Over the past 16 years he has pursued an interest in cellular pathology and bone biology within laboratories across the UK, Australia and America. Over this time he has won several awards, including the ASBMR Outstanding Contribution to the Pathophysiology of Osteoporosis Award and numerous Young Investigator Awards. He is co-chair of the ASBMR Committee for Young Investigators and serves on the editorial board of *Frontiers in Endocrinology of Ageing*. His work encompasses molecular mechanisms of bone cell interactions to advanced pre-clinical models including cancer-induced bone disease and age-related bone loss. His current studies focus on the role of lifespan-controlling factors, such as the sirtuin gene family, in the loss of skeletal integrity with age, identifying common regulatory links and potential therapeutic targets between the mechanisms which control longevity and the deterioration of the musculoskeletal system.

Peter Fratzl
Peter Fratzl is director at the Max Planck Institute of Colloids and Interfaces in Potsdam, Germany, and honorary professor of physics at Humboldt University, Berlin, and at Potsdam University. He received an engineering degree from the Ecole Polytechnique in Paris, France (1980), and a doctorate in Physics from the University of Vienna, Austria (1983). Before moving to Potsdam in 2003, he has been holding professor positions in materials physics at the Universities of Vienna and Leoben in Austria and been director of the Erich Schmid Institute of materials science of the Austrian Academy of Sciences.

Peter Fratzl's lab studies the relation between (hierarchical) structure and mechanical behaviour of biological materials, such as mineralized tissues, extracellular matrix, or plant cell walls, as well as bio-inspired composite materials. This is complemented by medically oriented research on osteoporosis and bone regeneration. Peter Fratzl has published more than 350 papers in journals and books, mostly on interdisciplinary materials science topics. He received several international awards for his work including the Max Planck Research Award 2008 from the Humboldt Foundation (together with Robert Langer, MIT) and the Leibniz Award 2010 of the German Science Foundation. In 2010, he was awarded an honorary doctorate from the University of Montpellier, France, and since 2007 he is foreign member of the Austrian Academy of Sciences.

Mary Goldring
Professor Goldring is Senior Scientist in the Research Division of the Hospital for Special Surgery and Professor of Cell and Developmental Biology, Weill Cornell Medical College in New York. Previously, she was principal investigator of research groups at the Massachusetts General Hospital and Beth Israel Deaconess Medical Center at Harvard Medical School, Boston, MA. Major contributions include the identification of the molecular mechanisms involved in matrix remodeling in osteoarthritis and development of in vitro models for studying human chondrocyte biology. Current work involves relating findings in mouse models of OA to aspects of the human disease. Her research is supported by grants from NIA and NIAMS. She is Co-

Editor of *Arthritis & Rheumatism* and Associate Editor of *Journal of Cellular Physiology*, *Arthritis Research and Therapy* and *Journal of Orthopedic Research*. She is 3rd Vice President of the ORS and a member of the Board of Directors of OARSI and has served on study sections for NIH, the Arthritis Foundation, OREF, and NASA and organizing committees of the 2005 and 2007 Cartilage Gordon Conferences.

Nick Higgins
Consultant Radiologist, University of Cambridge

John Kanis
Professor John A. Kanis is Emeritus Professor in Human Metabolism, and Director, World Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, UK. He is the President of the International Osteoporosis Foundation. Professor Kanis' research interests are largely related to disorders of skeletal metabolism including osteoporosis, Paget's disease of bone, hyperparathyroidism, renal osteodystrophy and neoplasia affecting the skeleton. Contributions to research include cell biology, histomorphometry of bone, assessment and treatment of bone disorders, guideline development, health technology assessment, epidemiology and health economics. He is the Editor of Osteoporosis International and serves on the editorial board of several journals. He is the author of more than 800 papers, chapters and books on bone disease and metabolism. His current major interest is in the development of risk assessment algorithms and the formulation of practice guidelines in many regions of the world.

Richard Keen
Consultant Rheumatologist and Honorary Senior Lecturer in Metabolic Bone Disease.
Dr Richard Keen graduated from St Mary's Hospital, London, UK in 1988. After general medical posts in London and Sheffield he started professional training in rheumatology, and during this time developed his specialist interest in osteoporosis. He is now Director of the Metabolic Bone Disease Unit at the Royal National Orthopaedic Hospital, Stanmore, UK. He also holds a honorary senior lecturer appointment at the Institute of Orthopaedics and Musculoskeletal Science, University College London, London.

Andy Pitsillides
Andy is a Professor in Skeletal Dynamics with interests spanning several areas of cell: matrix biology in the skeletal system. With growing emphasis on early mechano-dependent embryonic events, some of his research aims to establish how joints develop and how skeletal tissues adapt their structure. With a BSc and PhD (1988) awarded whilst at the Kennedy Institute, he later characterised synovial cell function in hyaluronan synthesis and described a novel role for nitric oxide in bone cells' mechanoadaptive response. He is Board Member of the London Matrix Group and British Society for Matrix Biology, Executive Editor of Cell Biochemistry and Function and Executive Committee Member of International Society for Hyaluronan Sciences.

Jonathan Reeve
Dr Reeve qualified in Medicine at Oxford and received further training at Guy's, the Hammersmith and Northwick Park. His research career began with Norman Veall, the 'father' of Nuclear Medicine, as a MRC Research Fellow at Northwick Park Hospital and Clinical Research Centre (CRC) Harrow, following an informal discussion of the mathematical modelling of calcium metabolism. At CRC he did the first clinical trial of teriparatide (parathyroid hormone), whose structure had recently been confirmed by John Potts and colleagues. This revealed it to have a strong bone building effect. After the MRC closed the CRC, he moved to Cambridge in 1994. He led the 30+ centre European Prospective Osteoporosis Study (EPOS) from 1993 with Alan Silman, which contributed to the much used FRAX tool for assessing fracture risk in osteoporosis. With Nigel Loveridge he also led a programme of research on the cellular and structural determinants of hip fracture risk, which led to collaboration with Chris Burgoyne and Tom Beck on the role of elastic instability (buckling) of the femoral neck cortex in this fracture. Study of the femoral osteocyte also began in 1994, first with its death and later with studies of secreted sclerostin, in collaboration with Socrates Papapoulos and colleagues. His current research interests include the genetic causes of fracture (as part of the GEFOS consortium) which grew out of EPOS and the EPIC-Norfolk study. He is a past Secretary and President of the Bone Research Society.

Graham Russell
Graham Russell (R G G Russell) graduated with first-class honours in Biochemistry from Cambridge University in 1962 and subsequently gained his PhD working in the MRC Unit in Leeds. After a fellowship in Switzerland he completed his medical degree at Oxford University. Following appointments at Bern University and then Harvard, he became Professor and Head of the Department of Human Metabolism and Clinical Biochemistry at Sheffield University in 1977. Under his leadership that department became established as a major international centre for the study of basic and clinical research into bone diseases. His research interests are in skeletal biology and disease, and he is author of more than 500 publications. In particular, his early work with Herbert Fleisch in Switzerland led to the discovery of the biological effects of bisphosphonates, and to their eventual successful clinical use in the treatment of bone disorders, including Paget's disease, cancer metastases in bone, and osteoporosis. His group later discovered how bisphosphonates act within cells as inhibitors of the mevalonate pathway.
He has held many prestigious offices, including the Presidency of the International Bone & Mineral Society (1998-2002), and he was a founding Trustee and subsequent Chairman of the Council of Management of the National Osteoporosis Society (UK). He was the Heberden Orator of the BRS in 1993 and was the recipient of the John Johnson Award of the Paget's Foundation (USA) in 1997. He has received the W F Neuman award of the American Society of Bone and Mineral Metabolism, the Gaillard award of the IBMS, and in 2008 he was elected a Fellow of the Royal Society of London (FRS).
From 2001-7 he was the Norman Collisson Professor of Musculoskeletal Sciences at Oxford University, and the first Director of the Botnar Research Centre. He is continuing his research as Professor of Musculoskeletal Pharmacology in Oxford and in the Mellanby Centre at Sheffield University.

Molly Stevens
Molly Stevens is currently Professor of Biomedical Materials and Regenerative Medicine and the Research Director for Biomedical Material Sciences in the Institute of Biomedical Engineering at Imperial College. She joined Imperial in 2004 after a Postdoctoral training in the field of tissue engineering with Professor Robert Langer in the Chemical Engineering Department at the Massachusetts Institute of Technology (MIT). In 2010 she received the Polymer International-IUPAC award for creativity in polymer science, the Rosenhain medal and the Norman Heatley Prize for Interdisciplinary research from the Royal Society of Chemistry. She has also recently been recognised by the TR100, a compilation of the top innovators, under the age of 35, who are transforming technology - and the world with their work. Her group is focussed on both high quality fundamental science and translation for human health. Research in regenerative medicine within her group includes the directed differentiation of stem cells, the design of novel bioactive scaffolds and new approaches towards tissue regeneration. She is the co-founder of RepRegen and InTiGen.

Jonathan Tobias
Jonathan Tobias is Professor of Rheumatology at the University of Bristol, and consultant rheumatologist at North Bristol Trust. Following undergraduate studies in medicine at Cambridge University and London University from where he qualified in 1984, he completed MD and PhD theses in bone biology in 1990 and 1994, at St George's Hospital Medical School in London. He moved to the University of

Bristol in 1995, and since 2008 has been based at the Musculoskeletal Research Unit at the Avon Orthopaedic Centre, Southmead Hospital. He manages a diverse research programme into the causes and treatment of osteoporosis, directs bone research within the Avon Longitudinal Study of Parents and Children, and has over 90 original peer-reviewed research publications in the bone field. He also has extensive clinical experience in treating patients with osteoporosis, and in running DXA-based osteoporosis diagnostic services. He has served on the editorial boards of *Journal of Bone and Mineral Research* and *Calcified Tissue International*, and on the Research and Education committee of the British Society for Rheumatology, the programme committee of the National Osteoporosis Society, and the research committee of the Arthritis Research UK. He is currently president of the Bone Research Society.

Toni Vidal-Puig

Dr Vidal-Puig obtained his medical degree from Valencia Medical School (Spain) before training in clinical endocrinology at Granada Medical School (Spain), where he obtained his PhD based on clinical and physiological studies of the relationship between insulin resistance and hyperandrogenism. The award of the Paul Dudley White Fellowship from the American Heart Association funded post-doctoral training at Harvard University, supporting his work with Dr David Moller and Prof Jeffrey Flier at the Beth Israel Hospital. Having published several key papers on the genetics and expression of PPARg in human disease states and been appointed Instructor in Medicine at Harvard, Dr Vidal-Puig further broadened his scientific horizons with experience in mouse transgenesis and knockout techniques in Prof Brad Lowell's group. In 2000 he moved to the University of Cambridge to establish his own laboratory and embark on the development of a programme based on genetically modified mouse models of metabolic diseases.

Dr Vidal-Puig is currently the Professor of Molecular Nutrition and Metabolism at Cambridge University and Honorary Consultant in Metabolic Medicine at Addenbrooke's Hospital, Cambridge. He is Deputy Director of the MRC Centre for Obesity and Related Diseases and Director of the Cambridge Phenomics Centre, a state-of-the-art centre that applies multidisciplinary approaches to murine phenotyping. His programme of research focuses on the molecular mechanisms of lipid-induced insulin resistance and on developing strategies to prevent the deleterious effects of lipids, specifically by modulating fatty acid oxidation and thermogenic mechanisms.

Richard Villar

Richard Villar is a consultant orthopaedic surgeon at the Cambridge Lea Hospital, Cambridge, UK. He is a specialist in surgery of the young adult hip. He has been undertaking hip arthroplasty for more than 15 years and has been performing hip arthroscopic surgery since 1988. In this latter field he wrote one of the first world texts on the subject and publishes and lectures extensively. He treats many premier national and international athletes. In addition to his clinical commitments, Mr Richard Villar has been the Assistant Editor of the British Journal of Bone and Joint Surgery, for whom he now produces a monthly podcast, and also runs a comprehensive Fellowship programme. He has been an elected member of the Council of the British Orthopaedic Association, an assessor for the General Medical Council and a Founding Member of the International Society for Hip Arthroscopy. Richard Villar has extensive overseas interests and lives near Cambridge in the United Kingdom. His leisure activities include mountaineering (he is a trained International Mountain Leader), Nordic skiing and classical guitar.

Keith Willett

Professor Keith Willett was appointed as the first National Clinical Director for Trauma Care on 1 April 2009.

He has extensive experience of trauma care and medical management and is Professor of Orthopaedic Trauma Surgery at the University of Oxford and continues to work as Honorary Consultant Orthopaedic Trauma Surgeon at the John Radcliffe Hospital, Oxford.

Keith is the co-founder of the unique consultant delivered Oxford Trauma Service established in 1993. In 2003 he founded the Kadoorie Centre for Critical Care Research and Education focusing on outcomes of treatment in the injured patient, and established the Oxford Trauma Research Group.

Keith has an extensive research portfolio and has published research relating to the care of the multiply injured patient, acetabular and pelvic fractures, fractures in the elderly, limb fracture surgery, fracture biomechanics, accident prevention and clinical outcome studies of orthopaedic trauma surgery techniques.

EXHIBITOR PROFILES

Amgen

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Amgen discovers, develops, manufactures, and delivers innovative human therapeutics. A biotechnology pioneer since 1980, Amgen was one of the first companies to realize the new science's promise by bringing safe, effective medicines from lab to manufacturing plant to patient. Amgen therapeutics have changed the practice of medicine, helping millions of people around the world in the fight against cancer, kidney disease, rheumatoid arthritis, bone disease, and other serious illnesses. With a deep and broad pipeline of potential new medicines, Amgen remains committed to advancing science to dramatically improve people's lives. To learn more about our pioneering science and vital medicines, visit

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Baxter BioSurgery offers biomaterials to advance surgical procedures and improve clinical and patient outcomes.

FLOSEAL is a high viscosity gel for haemostasis - effective on both soft and hard tissues - from oozing to brisk bleeding. It works at the end of the coagulation cascade and is effective also in heparinised patients.

ACTIFUSE is a silicate substituted, calcium phosphate bone graft substitute that accelerates bone formation. It can be used in a broad range of spinal and orthopedic procedures with appropriate stabilizing hardware.

TISSUCOL/TISSEEL is a physiological fibrin sealant designed to enhance tissue healing.

TISSUDURA is a collagen biomatrix for dura regeneration that is remodeled into a living neodura within 16 weeks after implantation.

GENTAFLEECE is a gentamycin-impregnated collagen fleece to be used as a topical haemostat on larger oozings in regions with a high risk of infection.

Bose

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The Bose ElectroForce Systems Group is a leading supplier of materials testing and durability simulation instruments to research institutions, universities, medical device companies, and engineering companies worldwide. ElectroForce test instruments help customers design better products and get them to market faster and more efficiently. ElectroForce test instruments provide exceptional dynamic performance and precision for a variety of testing applications by using proprietary linear motor technology, including: characterization of engineered materials, soft tissue and biomaterials; fatigue testing of components used in industrial and consumer applications; and durability simulation of medical devices, including stents, endovascular grafts, and orthopaedic implants.

Eli Lilly

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Eli Lilly and Company Limited is one of the world's largest research-based pharmaceutical companies, dedicated to creating and delivering innovative pharmaceutical healthcare solutions that enable people to live longer, healthier and more active lives. Our research and development efforts constantly strive to address the world's growing unmet medical needs in several different clinical areas.

For information about our products or services, please come and talk to us at the Lilly stand, or log on to the Eli Lilly website at www.lilly.com.

We look forward to meeting you.

Furlong Foundation

www.frcf.org.uk

The Furlong Research Charitable Foundation (FRCF) is a London based charity that supports a wide spectrum of research programmes within the field of Orthopaedic Science. This ranges from general scientific programmes such as Molecular Genetic & Functional Analysis of Genes Harbouring Risk for Primary Osteoarthritis to controversial areas of research such as metal-on-metal bearing surfaces.

The Foundation also organises and co-sponsors many educational events, courses and symposia to further the training of surgeons, scientists and engineers throughout the country.

GE Healthcare LUNAR

www.ge.com

GE Healthcare, Lunar is a leading densitometry equipment partner worldwide. We are dedicated to develop innovative and productive bone assessment systems to help you diagnose osteoporosis.

GE provides a complete bone densitometry product portfolio to make bone densitometry accessible to as many patients as possible. We offer high-definition technology to x-ray free fracture risk assessment….all with proven precision that is crucial for accurate patient bone assessment and treatment monitoring.

Heraeus Medical

www.heraeus-medical.com

Heraeus Medical is one of the leading companies in the field of bone cement, biomaterials and bio surgery for elective orthopaedic and trauma surgery. As a result, the company makes an essential contribution by improving the surgical outcome in bone and joint surgery leading to an improvement in the patients' health-related quality of life.

The key product PALACOS®, is regarded as the Gold Standard amongst bone cements, with a history of clinical efficacy spanning five decades. In the field of bio-surgery, Heraeus develops and offers innovative anti-infective coatings for medical implants.

Immunodiagnostic Systems (IDS)

www.idsplc.com

IDS excels at providing scientifically advanced solutions that allow laboratories to efficiently perform and confidently report results for important specialty markers in the areas of bone, cartilage, and growth. IOF and IFCC experts recommend that s-PINP and s-CTX are used as reference analytes for bone turnover markers in observational and intervention studies. Together with the co-specific 25 Vitamin D, the s-PINP and s-CTX can be consolidated onto the automated IDS-iSYS system for a complete bone turnover and nutritional status.

Please come and visit us at the IDS stand to discuss:

- Our Vitamin D tests;
- How our bone and growth assays can support your research; and
- Specialty ELISA kits from our partners.

Instron

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Instron is a leading provider of testing equipment for the material testing and structural testing markets. Instron's products test the mechanical properties and performance of various materials, components and structures in a wide array of environments. A global company providing single-source convenience, Instron is a full-service materials testing company that manufactures and services testing instruments, systems, software and accessories.

Instron's proficiency in designing and building testing systems to evaluate materials ranging from the most fragile filament to advanced alloys, affords Instron's customers a comprehensive resource for all their research, quality and service-life testing requirements. Information is also available on the company's enhanced website at www.instron.com.

Materlialise
www.materialise.com
Since 1990 Materialise has been dedicated to offering you a total solution for advancing your biomedical research and development. The **Mimics Innovation Suite** is a platform
That encompasses a variety of products and services tailored to challenges encountered
in the biomedical field. From scan data to visualization and beyond, our solutions are powerful tools for taking your R&D to the next level.

The **Mimics Innovation Suite is** FDA 510(k) and CE approved.
Mimics and 3-matic are developed at Materialise within an ISO 9001 certified quality system or ISO 13485 for medical software.

Medtronic
www.medtronic.co.uk
Our Spinal and Biologics business is committed to advancing the treatment of spinal conditions, and as the global leader in today's Spinal and Biologics market, we lead the way in innovative solutions to treat the Spine.

A crucial element to this innovation and market leadership is that our business is committed to development of new technologies through collaboration. We work with world-renowned surgeons, researchers and other innovative partners to offer state-of-the-art products and technologies.

Orthovita UK Ltd
www.orthovita.com
Orthovita is an established leader in the product development, manufacturing and marketing of innovative Orthobiologics materials. The company portfolio includes proven safe and effective products for procedures in Spine and Orthopaedics, involving fusion, regeneration and fixation of human bone.

Vitoss® and Vitoss BA® Bone Graft Substitute has one of the most robust data packages on the market with more than 18 human clinical spine studies in over 800 patients that includes Level I Human Clinical Data.

Cortoss® Bone Augmentation Material is a bioactive augmentation material specifically licensed for vertebral augmentation and screw fixation. Cortoss® offers superior intra-operative handling due to its unique composition.

Pathway Diagnostics
www.pathwaydiagnostics.com
Pathway Diagnostics Ltd is a specialist in the field of bone, cartilage and mineral metabolism immunoassays and is the exclusive distributor in the UK and Ireland of both the MicroVue® range of Bone Marker assay kits from Quidel and Cartilage assays kits from Ibex Diagnostics.
The MicroVue® range of ELISA assays includes a highly specific Bone Alkaline Phosphatase (BAP) assay, serum Osteocalcin, Collagen Type –1 C-Terminal propeptide (CICP), DPD and Total DPD, PYD, TRAP5b, OPG, Helical Peptide and YKL-40, as well as a chromogenic Creatinine assay and other research reagents.

Ibex Diagnostics specialises in cartilage assay kits including C2C and C1,2C ELISA's for cartilage degradation (serum & urine), CPII and CS-846 ELISA's for cartilage formation (serum & urine).

Details of new products at the BRS meeting include

- TECO Sclerostin ELISA assay
- New Coll2-1 and Coll2-1NO2 Cartilage assays

Pathway Diagnostics also markets an extensive range of metabolic disease marker assays, autoimmune assays in multiplex and ELISA format, Complement test and research reagents, and multiplex human cytokine assays.

Ranier
www.ranier.co.uk
Ranier is based in Cambridge, UK and its lead product, Cadisc™ -L, is an entirely polyurethane, load sharing, replacement lumbar disc. It is designed to mimic both the functionality & biomechanics of the natural lumbar disc, providing both physiological range and physiological quality of motion. Cadisc™ -L has no articulating surfaces and no metal endplates for full MRI compatibility; the bone contacting surfaces have a Calcium Phosphate coating and ribs for fixation. Ranier is also developing an anatomical cervical disc, Cadisc™ –C which shares the kinematic & load sharing advantages of its lumbar stable mate.

Servier Laboratories
www.servier.co.uk

SkyScan/e2v
www.e2v.com
www.skyscan.be
SkyScan specializes in the development and manufacturing of X-ray microtomography (micro-CT) systems for the non-destructive investigation of an object's internal microstructure. The standard commercial microtomography systems from SkyScan today reach a spatial resolution in the submicron range. A comprehensive range of systems covering a wide range of budgets in both in-vivo and in-vitro areas is backed by a dedicated team of specialists in the applications of microCT technology in.biological and other fields. SkyScan aims to bring to customers the highest possible level of support and the best instrument quality. To this end, we have a continuous development program for both our hardware and our comprehensive quantitative software analysis suite. Responding to demand from the growing community of micro-CT users, we strive to keep our research and development at the cutting edge of non-destructive 3D microscopy.

Vertec Scientific
www.vertec.co.uk
Now established in the UK for over thirty years, Vertec Scientific, based in Silchester, near Reading, is a leading distributor of world class systems for diagnostic imaging and radiotherapy in the UK. The Company's portfolio features intraoperative x-ray c-arms and DXA from Hologic, radiotherapy patient immobilisation systems from Klarity Medical, ultrasound from Siemens, portable CT from Neurologica, virtual colonoscopy and cardiology from Viatronix, radiation protection from Scanflex, Gafchromic dosimetry film, the innovative DR x-ray system Opera Swing from GMM and the newly launched, vet digital x-ray system from DRGEM Corp, plus Picsara, a highly innovative multi-image management system.

Zwick Testing
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Worldwide demands in the booming medical sector have resulted in the growth of more complex materials and component testing. Increasing regulations regarding certification, traceability and auditing of data have introduced additional challenges to laboratory process management. As a result, more and more companies are turning to Zwick to provide a complete solution, due to its extensive experience in the industry.

Zwick pays close attention to the market needs including the introduction of new products, applications and customer support. It welcomes co-operation between materials testing experts and medical specialists in order to provide test environments which replicate as closely as possible, conditions found in the human body.