1st Joint Meeting

OF THE

Bone Research Society
(formerly Bone and Tooth Society)

AND THE

British Orthopaedic Research Society

5-6 July 2006

SOUTHAMPTON, UK

Contents

BORS President
Bruce Caterson
BRS President
Richard Eastell
Local Organisers
Richard Oreffo
Trudy Roach
Cyrus Cooper
Elaine Dennison

www.brs-bors-2006.org

Bone Research Society

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 presentations at the annual meeting are traditionally a balance between clinical and laboratory-based studies. The participation of young scientists and clinicians is actively encouraged.

Committee 2006

President: Richard Eastell (Sheffield)
President Elect: Jonathan Reeve (Cambridge)
Secretary: Tim Arnett (London)
Treasurer: Jonathan Tobias (Bristol)
Mark Cooper (Birmingham)
Peter Croucher (Sheffield)
Bronwen Evans (Cardiff)
Miep Helfrich (Aberdeen)
Richard Keen (London)
Richard Oreffo (Southampton)

Meeting Organiser

Janet Crompton
Tel: +44 (0)1453 549929
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E-mail janet@janet-crompton.com

Membership Enquiries

BioScientifica Ltd
Tel: +44 (0)1454 642200
Fax: +44 (0)1454 642222
E-mail: info@endocrinology.org

FUTURE BRS MEETINGS

21 November 2006

Clinical Meeting, Cambridge
Organiser: Gavin Clunie
(gavin.clunie@ipswichhospital.nhs.uk)

3-5 July 2007

Annual Meeting, Aberdeen
Organiser: David Reid
Deadline for abstracts: 16 February 2007
Details from Janet Crompton as above

BONE
RESEARCH SOCIETY

www.brsoc.org.uk

British Orthopaedic Research Society

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 2006

President: Bruce Caterson (Cardiff)
Secretary: Carlos Wigderowitz (Dundee)
Treasurer: Gang Li (Belfast)
Roger Bayston (Nottingham)
Mark Birch (Newcastle upon Tyne)
Carol Evans (Manchester)
David Marsh (London)
Andrew McCaskie (Newcastle upon Tyne)
Tony Miles (Bath)

Membership Enquiries

Margaret E Aitken
c/o Mr Carlos Wigderowitz,
Honorary Secretary BORS

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Arcana ossium quis explorat – B.O.R.S.

www.borsoc.org.uk

Awards, Sponsors and Other Supporters

Awards were made by both societies according to marks given to blinded abstracts during the independent review process.

Bone Research Society Award Winners

BRS Scholarships

A Brandao-Burch (London): OC24: Activation of human osteoclasts by capsaicin

J Crockett (Aberdeen): OP6: Mutations in the rank signal peptide alter the subcellular localisation of rank and prevent ligand-dependent activation of NFkappabeta

G Kogianni (Edinburgh): OC26: In vivo stimulation of bone resorption by apoptotic osteocytes

G Spencer (York): OC3: Functional cholinergic signalling mechanisms and the central regulation of human mesenchymal stem cells

Young Investigator Awards

N Harvey (Southampton): OC37: MRNA expression of human placental calcium transporter (PMCA3) predicts intrauterine bone mineral accrual in the offspring

M Javaid (Southampton): OC38: Growth in infancy and childhood predicts hip fracture risk in late adulthood in men and women

British Orthopaedic Research Society Award Winners

B Bolland (Southampton): P1: Translation from laboratory to theatre: augmentation of impacted allograft with human bone marrow stromal cells

P Buddhdev (Stanmore): P34: Cup inclination angle is positively correlated with whole blood concentrations of cobalt and chromium ions after metal-on-metal hip resurfacing

X Chen (Belfast): OC2: Survival of xenogeneic bone marrow-derived mesenchymal stem cells in a xeno-transplantation model

P Heaton-Adegbile (London): P5: A new hip simulator for in-vitro fatigue testing of implanted acetabula

G Kirmizidis (Newcastle-upon-Tyne): OC34: Biomaterial surface architecture dictates cell:cell interactions and regulates osteoblast differentiation

M Quaye (London): P27: A comparison of bone fragment displacement in two distal radius ORIF techniques

A Unnithan (Worthing): OP5: A novel approach to creating a bio mimetic hydroxyapatite coating on fine surface features to enhance osseointegration of external fixation screws

P Vadillo (Edinburgh): P6: Three-dimensional polycaprolactone scaffolds for tissue engineering

R Weaver (Hatfield): P61: Novel application of PQCT to standardise synovial fluid biomarker concentrations

Major Sponsors:

Alliance for Better Bone Health (Procter and Gamble Pharmaceuticals and sanofi aventis)
Eli Lilly
Merck Sharp & Dohme
Pfizer
Roche/GlaxoSmithKline
Servier
Shire Pharmaceuticals

Other Supporters:

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Orthodynamics
Oxford Biosystems
Promocell
Regenerative Medicine
Stryker Howmedica
Technoclone
Zimmer

Outline Programme
Wednesday 5 July

	NUFFIELD THEATRE	GARDEN COURT	LECTURE THEATRE H
08.30			
09.00		Registration/ poster hanging/ coffee	
09.30			
10.00			
10.30	SYMPOSIUM: Stem cells: basic science and therapeutic potential		
11.00			
11.30	ORAL COMMUNICATIONS		
12.00			
12.30			
13.00	BORS AGM and Awards	LUNCH and poster viewing	
13.30			
14.00	SYMPOSIUM: Bioengineering		SYMPOSIUM: Genetics and epigenetics
14.30	ORAL COMMUNICATIONS		
15.00		TEA	
15.30	SYMPOSIUM: Bone and Joint Decade		SYMPOSIUM: Matrix proteins in bone formation
16.00			
16.30	ORAL COMMUNICATIONS		ORAL COMMUNICATIONS
17.00			
17.30			
18.00			
18.30		JOINT POSTER SESSION with wine and cheese	
19.00			
19.30			
20.00			
20.15		CONFERENCE DINNER	

Stem cells: basic science and therapeutic potential

Stephen Minger (London, UK): EMBRYONIC STEM CELLS

Christopher Evans (Boston, USA): GENE THERAPIES IN MUSCULOSKELETAL DISEASE

Genetics and epigenetics

John Loughlin (Oxford, UK): GENETICS OF OSTEOARTHRITIS

John Newell-Price (Sheffield, UK): DNA METHYLATION AND THE SILENCING OF GENE EXPRESSION

Bioengineering

John Fisher (Leeds, UK): WEAR OF TOTAL JOINT REPLACEMENTS

Bone and Joint Decade

Michael Adams (Bristol, UK): WHAT IS INTERVERTEBRAL DISC DEGENERATION, AND HOW DOES IT AFFECT VERTEBRAE?

Matrix proteins in bone formation

Lynda Bonewald (Kansas City, USA): OSTEOCYTE MODIFICATION OF EXTRACELLULAR MATRIX

Marian Young (Bethesda, USA): SMALL LEUCINE RICH PROTEOGLYCANS IN THE SKELETON

LECTURE THEATRE A
CLINICAL CASES

Outline Programme
Thursday 6 July

	NUFFIELD THEATRE	GARDEN COURT	LECTURE THEATRE H
08.30			
09.00	**SYMPOSIUM: Fetal origins of skeletal development**		**SYMPOSIUM: Nanotechnology and imaging**
09.30	**ORAL COMMUNICATIONS**		**ORAL COMMUNICATIONS**
10.00			
10.30		**COFFEE and poster viewing**	
11.00			
11.30	**ORAL POSTERS**		
12.00			
12.30			
13.00	**BRS AGM AND AWARDS**	**LUNCH and poster viewing**	
13.30			
14.00	**SYMPOSIUM: Clinical Disorders - Paediatric/Metabolic bone disease**		
14.30			
15.00			
15.30		**TEA**	**LECTURE THEATRE J**
16.00	**CLINICAL WORKSHOP**		**SYMPOSIUM: Musculoskeletal regeneration**
16.30			**ORAL COMMUNICATIONS**
17.00			
17.30			
18.00			
18.30			
19.00			
19.30			
20.00			
20.15			

Fetal origins of skeletal development

Cyrus Cooper (Southampton, UK): DEVELOPMENTAL ORIGINS OF OSTEOPOROTIC FRACTURES

Jon Tobias (Bristol, UK): DETERMINANTS OF BONE GROWTH IN CHILDHOOD: INSIGHTS FROM THE ALSPAC COHORT

Nanotechnology and imaging

Laurent Bozec (London, UK): SKELETAL TISSUES AT THE NANOSCALE

Clinical Disorders - Paediatric/ Metabolic bone disease

Nick Bishop (Sheffield, UK): BONE FRAGILITY IN CHILDREN

Ian Reid (Auckland, New Zealand): THE LINKS BETWEEN BODY COMPOSITION AND BONE

David Marsh (London, UK): FUTURE MANAGEMENT OF FRACTURE RISK

Clinical Workshop

Richard Keen (London, UK): NICE GUIDELINES

John Kanis (Sheffield, UK): RISK ASSESSMENT

Richard Eastell (Sheffield, UK): OSTEOPOROSIS TREATMENT IN THE BIOLOGICS ERA

Frazer Anderson (Southampton, UK): VITAMIN D: WHO NEEDS SUPPLEMENTATION AND WITH WHAT?

Musculoskeletal regeneration

Alicia El-Haj (Keele, UK): THERAPEUTIC CELL THERAPY AND TISSUE ENGINEERING: NOVEL MAGNETIC STATEGIES FOR GROWTH CONTROL AND DELIVERY

Programme
Wednesday 5 July

09.00 **GARDEN END OF GARDEN COURT**

Registration/poster hanging/coffee

09.50 **NUFFIELD THEATRE**

Welcome and introduction

Philip Nelson, Deputy Vice-Chancellor, University of Southampton

10.00 **NUFFIELD THEATRE**

SYMPOSIUM
Stem cells: basic science and therapeutic potential

Chairs:
Richard Oreffo (Southampton, UK)
David Marsh (Stanmore, UK)

10.00 IS1 EMBRYONIC STEM CELLS
Stephen Minger (London, UK)

10.30 IS2 GENE THERAPIES IN MUSCULOSKELETAL DISEASE
Chris Evans (Boston, USA)

11.00 **NUFFIELD THEATRE**

Oral Communications

Chairs:
Bruce Caterson (Cardiff, UK)
Jonathan Reeve (Cambridge, UK)

11.00 OC1 CLONAL PLASTICITY AND IN VIVO BONE FORMATION OF HUMAN FETAL FEMUR-DERIVED CELLS
SH Mirmalek-Sani[1], RS Tare[1], HI Roach[1], SM Morgan[1], DI Wilson[2], NA Hanley[2], ROC Oreffo[1]*
[1]Bone and Joint Research Group, Developmental Origins of Health and Disease Division;[2]Human Genetics Division, University of Southampton, SO16 6YD, UK

11.10 OC2 SURVIVAL OF XENOGENEIC BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN A XENO-TRANSPLANTATION MODEL
X Chen, G Li*
Musculoskeletal Research Unit, School of Biomedical Sciences, Musgrave Park Hospital, Queen's University Belfast, Belfast, BT9 7JB, UK

11.20 OC3 FUNCTIONAL CHOLINERGIC SIGNALLING MECHANISMS AND THE CENTRAL REGULATION OF HUMAN MESENCHYMAL STEM CELLS
MJ Hoogduijn, GJ Spencer, PG Genever*
Biomedical Tissue Research, University of York, York, UK

11.30 OC4 CAN MESENCHYMAL STEM CELLS PLAY A ROLE IN INCREASING NEW BONE FORMATION?
M Korda, G Blunn, N Little, J Hua
UCL, London

11.40 OC5 EXCESSIVE LOCAL GLUCOCORTICOID GENERATION MAY EXPLAIN THE IDIOPATHIC OSTEOPOROSIS OF GLYCOGEN STORAGE DISEASE TYPE 1A
A Ahmed, R Crook, EH Rabbitt, E Elias, PM Stewart, EA Walker, MS Cooper*
University of Birmingham, Birmingham, UK

11.50 OC6 THE DETECTION OF HUMAN EMBRYONIC AND ADULT STEM CELL SURVIVAL IN IMMUNE DEFICIENT AND IMMUNE COMPETENT RAT MODELS OF BONE REPAIR
SN Racey[1], JL Tremoleda[1], D Wojtacha[2], N Khan[1], J McWhir[2], AHRW Simpson[1], BS Noble[1]*
[1]Musculoskeletal Tissue Engineering Collaboration (MTEC), University of Edinburgh, Medical school, Edinburgh, UK;[2]Gene Function and Development, Roslin Institute. Roslin, UK

12.00 OC7 MILD PRIMARY HYPERPARATHYROIDISM IS ASSOCIATED WITH LOW BMD BUT NOT FRACTURE RISK, QUALITY OF LIFE OR MORTALITY IN COMMUNITY DWELLING ELDERLY WOMEN
E McCloskey, D deTakats, M Beneton, J Cliffe, L Reaney, C McGurk, D Charlesworth, T Jalava, J Kanis*
University of Sheffield, Sheffield, UK

12.10 OC8 ATDC5: AN IDEAL CELL LINE FOR STUDYING CHONDROCYTE DIFFERENTIATION AND MODELLING CARTILAGE FORMATION
RS Tare[1], R Forsey[2], JB Chaudhari[2], ROC Oreffo[1]*
[1]Bone & Joint Research Group, University of Southampton, Southampton, SO16 6YD, UK;[2]Department of Chemical Engineering, University of Bath, Bath, BA2 7AY, UK

12.20 OC9 ZOLEDRONATE REDUCES EARLY ACUTE BONE LOSS AT THE HIP FOLLOWING SPINAL CORD INJURY
JS Bubbear[1], A Gall[1], FRI Middleton[1], M Ferguson-Pell[2], RW Keen[1,3]*
[1]Royal National Orthopaedic Hospital, Stanmore, UK;[2]Aspire Centre for Disability Services, Stanmore, UK;[3]Institute of Orthopaedics & Musculoskeletal Science, University College Hospital, London, UK

12.30 **NUFFIELD THEATRE**

BORS AGM and awards

12.30 **GARDEN END OF GARDEN COURT**

LUNCH and poster viewing

13.30 **LECTURE THEATRE H**

SYMPOSIUM
Genetics and epigenetics

Chairs:
Nigel Arden (Southampton, UK)
Trudy Roach (Southampton UK)

13.30 IS3 GENETICS OF OSTEOARTHRITIS
John Loughlin (Oxford, UK)

14.00 IS4 DNA METHYLATION AND THE SILENCING OF GENE EXPRESSION
John Newell-Price (Sheffield, UK)

13.30 NUFFIELD THEATRE

SYMPOSIUM
Bioengineering

Chairs:
Alicia El-Haj (Keele, UK)
Alan Goodship (Stanmore, UK)

13.30 IS5 WEAR OF TOTAL JOINT REPLACEMENTS
John Fisher (Leeds, UK)

Oral Communications

Chairs:
Alicia El-Haj (Keele, UK)
Alan Goodship (Stanmore, UK)

14.00 OC10 A FINITE ELEMENT STUDY OF A HIP RESURFACING ARTHROPLASTY WITH AND WITHOUT A STEM
JP Little, DW Murray, HS Gill*
Oxford Orthopaedic Engineering Collaboration, University of Oxford, Oxford, UK

14.10 OC11 CHROMOSOMAL ABERRATIONS IN PATIENTS WHO HAVE HAD METAL-ON-METAL HIP BEARINGS IN-SITU FOR IN EXCESS OF 30 YEARS
E Dunstan[1], D Ladon[2], P Whittingham-Jones[1], S Cannon[1], P Case[2], T Briggs[1]*
[1]Royal National Orthopaedic Hospital, Stanmore; [2]Bristol Implants Research Center, Bristol

14.20 OC12 THE FRICTION BEHAVIOUR DURING THE WEAR OF METAL ON METAL HIP JOINTS WITH DIFFERENCE CLEARANCE
XQ Hu, A Taylor, M Tuke*
Finsbury Orthopaedics Ltd, Leatherhead, Surrey, UK

14.30 GARDEN END OF GARDEN COURT

TEA

15.00 LECTURE THEATRE H

SYMPOSIUM
Matrix proteins in bone formation

Chairs:
Miep Helfrich (Aberdeen, UK)
Nigel Loveridge (Cambridge, UK)

15.00 IS6 OSTEOCYTE MODIFICATION OF EXTRACELLULAR MATRIX
Lynda Bonewald (Kansas City, USA)

15.30 IS7 SMALL LEUCINE RICH PROTEOGLYCANS IN THE SKELETON
Marian Young (Bethesda, USA)

15.00 LECTURE THEATRE A

Clinical Cases

Chairs:
Richard Keen (London, UK)
Mike Adams (Bristol, UK)

15.00 CC1 COMPARISON OF THE USE OF HUMERUS INTRAMEDULLARY NAIL AND DYNAMIC COMPRESSION PLATE FOR THE MANAGEMENT OF DIAPHYSEAL FRACTURES OF HUMERUS: A PROSPECTIVE RANDOMISED STUDY
M Changulani, UK Jain*
Department of Trauma and Orthopedics, University Hospital, King George Medical College, Lucknow, Northern India

15.15 CC2 A BIOMECHANICAL STUDY COMPARING 6.5MM CANCELLOUS SCREWS AND 3.5MM CORTICAL SCREWS FOR DEPRESSED TIBIAL PLATEAU FRACTURES
S Patil[1], A Mahon[1], S Green[2], I Mcmurtry[1], A Port[1]
[1]James Cook University Hospital; [2]University of Durham

15.30 CC3 AUTOSOMAL DOMINANT CARPAL TARSAL OSTEOLYSIS: RESULTS OF TREATMENT WITH PAMIDRONATE
RD Gilbert, M Collins, J Fairhurst*
Southampton General Hospital

15.45 CC4 OSTEOGENIC OSTEOMALACIA ASSOCIATED WITH AN ETHMOIDAL SINUS TUMOUR
ZA Cole, EM Dennison, NK Arden*
MRC Epidemiology Resource Centre, Southampton General Hospital, Tremona Road, SO16 6YD, UK

15.00 NUFFIELD THEATRE

Symposium:
Bone and Joint Decade

Chairs:
Hamish Simpson (Edinburgh, UK)
Carlos Wigderowitz (Dundee, UK)

15.00 IS8 WHAT IS INTERVERTEBRAL DISC DEGENERATION, AND HOW DOES IT AFFECT VERTEBRAE?
Michael Adams (Bristol, UK)

Oral Communications

Chairs:
Hamish Simpson (Edinburgh, UK)
Carlos Wigderowitz (Dundee, UK)

15.30 OC13 LONG-BONE REGENERATION USING GUIDING MEMBRANES: CRYOPRESERVED ARTERIAL ALLOGRAFTS VERSUS SYNTHETIC MEMBRANES
MA Suarez-Suarez[1,2], M Alvarez-Rico[2], F Ferrero-Manzanal[2], P Menendez-Rodriguez[3], A Meana-Infiesta[4], J deCos-Juez[2], JC deVicente-Rodriguez[2,3], A Murcia-Mazon[1,2]*
[1]Cabueñes-Gijon Hospital, Asturias, Spain; [2]Oviedo University, Asturias, Spain; [3]Hospital Central, Asturias, Spain; [4]Centro Comunitario de Sangre y Tejidos, Asturias, Spain

15.40 OC14 ESTIMATE OF THE FUNCTIONAL IMPROVEMENT IN THUMB CMC JOINT OSTEOARTHRITIS AFTER A SINGLE INTRA-ARTICULAR STEROID INJECTION
M Khan, D Derham, M Waseem*
Department of Orthopaedics Macclesfield General Hospital,Victoria Road, Macclesfield, UK

15.50 OC15 DETECTION OF MATRIX METALLOPROTEINASES IN PRIMARY FROZEN SHOULDERS
IDM Brown[1], IG Kelly[1], Prof IB McInnes[2]*
[1]Glasgow Royal Infirmary, Glasgow, UK;[2]Centre for Rheumatic Diseases, University of Glasgow, UK

16.00 OC16 FUNCTIONAL IONOTROPIC GLUTAMATE RECEPTORS IN HUMAN FIBROBLAST-LIKE SYNOVIOCYTES MODULATE IL-6 AND MMP-2 EXPRESSION
*SJ Flood[1], R Parri[2], A Williams[3], VC Duance[1], DJ Mason[1]**
[1]Connective Tissue Biology Laboratories, School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3US, UK;[2]School of Life and Health Sciences Aston University Birmingham B4 7ET UK; [3]Department of Rheumatology,Cardiff University Heath Park,Cardiff CF14 4XN,UK

16.10 OC17 SEVERE MECHANICAL LOADING CAN CAUSE CERVICAL INTERVERTEBRAL DISCS TO PROLAPSE
P Pollintine, D Skrzypiec, P Dolan, MA Adams*
Department of Anatomy, University of Bristol, Bristol, UK

16.20 OC18 EVIDENCE FOR INVOLVEMENT OF A FACTOR X IN THE PROPAGATION OF THE OSTEOARTHRITIC PHENOTYPE
K Hashimoto[1,2], N Yamada[2], S Kokubun[2], HI Roach[1]*
[1]Bone and Joint Research Group, University of Southampton, Southampton, UK; [2]Department of Orthopaedics, Tohoku University, Sendai, Japan

16.30 OC19 DEFICIENCY OF THE SPECIFIC IMMUNE SYSTEM ENHANCES FRACTURE REPAIR IN-VIVO
MS Gaston, BS Noble, AHRW Simpson*
Musculoskeletal Tissue Engineering Consortium, University of Edinburgh, Chancellor's Building, 49 Little France Cresent, Edinburgh, UK

16.40 OC20 PRIMARY FROZEN SHOULDER THAWED
M Bains[1], S Lambert[2], V Mudera[1]*
[1]UCL, London, UK;[2]Royal National Orthopaedic Hospital, Stanmore, UK

16.50 OC21 HOW DO BMD, FRACTURE SEVERITY AND CEMENT TYPE INFLUENCE THE BIOMECHANICAL EFFECTS OF VERTEBROPLASTY?
J Luo[1], D Skrzypiec[1], P Pollintine[1], MA Adams[1], DJ Annesley-Williams[2], P Dolan[1]*
[1]Department of Anatomy, University of Bristol, Bristol, UK;[2]Queen's Medical Centre, Nottingham, UK

16.00 LECTURE THEATRE H

Oral Communications

Chairs:
Tim Arnett (London, UK)
Nick Harvey (Southampton, UK)

16.00 OC22 OSTEOCYTES REPAIR FOLLOWING MICRODAMAGE INJURY IN VITRO
MY Loqman, G Kogianni, AHRW Simpson, BS Noble*
Edinburgh, UK

16.10 OC23 INSIGHT INTO THE FUNCTION OF BCL-2-ASSOCIATED ATHANOGENE-1 ANTI-APOPTOTIC PROTEIN DURING CHONDROCYTE DEVELOPMENT AND DIFFERENTIATION
RS Tare[1], PA Townsend[2], G Packham[3], ROC Oreffo[1]*
[1]Bone & Joint Research Group; [2]Human Genetics; [3]Cancer Sciences, University of Southampton, Southampton SO16 6YD, UK

16.20 OC24 ACTIVATION OF HUMAN OSTEOCLASTS BY CAPSAICIN
A Brandao-Burch, TR Arnett*
Department of Anatomy and Developmental Biology, University College London, London, UK

16.30 OC25 AN OSTEOPROTEGERIN-LIKE PEPTIDOMIMETIC (OP3-4) INHIBITS OSTEOCLASTIC BONE RESORPTION AND OSTEOLYTIC BONE DISEASE IN MULTIPLE MYELOMA
DJ Heath[1], K Vanderkerken[2], X Cheng[3], O Gallagher[1], M Prideaux[1], R Murali[2], PI Croucher[1]*
[1]University of Sheffield, UK; [2]Free University Brussels, Belgium; [3]University of Pennsylvania, USA

16.40 OC26 IN VIVO STIMULATION OF BONE RESORPTION BY APOPTOTIC OSTEOCYTES
G Kogianni, V Mann, BS Noble*
Musculoskeletal Tissue Engineering Collaboration (MTEC), University of Edinburgh, Medical School, Edinburgh, UK

16.50 OC27 A NOVEL ROLE FOR IL-23 IN OSTEOCLAST DEVELOPMENT
L Chen, DP Aeschlimann, XQ Wei*
Department of Dental Health and Biological Sciences, Cardiff, UK

17.00 BREAK

18.00-20.00 GARDEN END OF GARDEN COURT

Poster session with wine and cheese

18.00 Odd numbered posters to be manned

19.00 Even numbered posters to be manned

20.15-24.00 GARDEN COURT

Conference dinner

Programme
Thursday 6 July

08.30 **NUFFIELD THEATRE**

SYMPOSIUM
Fetal origins of skeletal development

Chairs:
Richard Eastell (Sheffield, UK)
Ian Reid (Auckland, New Zealand)

08.30 IS9 DEVELOPMENTAL ORIGINS OF OSTEOPOROTIC FRACTURES
Cyrus Cooper (Southampton, UK)

09.00 IS10 DETERMINANTS OF BONE GROWTH IN CHILDHOOD: INSIGHTS FROM THE ALSPAC COHORT
Jon Tobias (Bristol, UK)

09.30 **NUFFIELD THEATRE**

Oral Communications

Chairs:
Bronwen Evans (Cardiff, UK)
Lynda Bonewald (Kansas City, USA)

09:30 OC37 MRNA EXPRESSION OF HUMAN PLACENTAL CALCIUM TRANSPORTER (PMCA3) PREDICTS INTRAUTERINE BONE MINERAL ACCRUAL IN THE OFFSPRING
NC Harvey[1], R Martin[2], MK Javaid[1], P Taylor[3], JR Poole[1], EM Dennison[1], HM Inskip[1], KM Godfrey[1,2], C Cooper[1], RM Lewis[2]*
[1]MRC Epidemiology Resource Centre, Southampton, UK; [2]Centre for the Developmental Origins of Health and Disease, University of Southampton, Southampton, UK; [3]Medical Physics and Bioengineering, Southampton General Hospital, Southampton, UK

09:40 OC38 GROWTH IN INFANCY AND CHILDHOOD PREDICTS HIP FRACTURE RISK IN LATE ADULTHOOD IN MEN AND WOMEN
MK Javaid[1], JG Eriksson[2], M Välimäki[2], E Kgjantie[2], T Forsen[2], C Osmond[1], DJ Barker[1], C Cooper[1]*
[1]MRC Epidemiology Resource Centre, Southampton, UK; [2]The National Public Health Institute, Helsinki, Finland

09:50 OC39 A RATIONALE FOR TREATING LEG LENGTH DISCREPANCY USING PHOTODYNAMIC THERAPY
SK Bisland[1], C Johnson[1], M Diab[2], BC Wilson[1], S Burch[1,2]*
[1]Princess Margaret Hospital, Ontario Cancer Institute, University Health Network, Toronto, Ontario, M5G 2M9, Canada ;[2]University of California, San Francisco, Department of Orthopaedic Surgery, San Francisco, CA USA

10:00 OC40 REMODELLING CLUSTERS REDUCE LOCAL BONE STRAIN AND FAVOUR CANAL MERGING
D Thomas, J Clement, J Reeve, N Loveridge
School of Dental Science, University of Melbourne, Australia & University of Cambridge UK

10:10 OC41 ACTIVATION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR DELTA (PPARDELTA) INCREASES PERIOSTEAL BONE FORMATION
K Still[1], MJ Perry[2], P Grabowski[1], NJ Bishop[1]*
[1]Academic Unit of Bone Biology/Child Health, University of Sheffield; [2]Department of Anatomy, University of Bristol

10:20 OC42 NEW SCAFFOLDS FOR SKELETAL REGENERATION: STIMULATING ANGIOGENESIS AND OSTEOGENESIS
J Kanczler[1], J Barry[2], P Ginty[2], W Sebald[3], S Howdle[2], K Shakesheff[2], ROC Oreffo[1]*
[1]University of Southampton, UK;[2]University of Nottingham, UK; [3]University of Wurzburg, Germany

08.30 **LECTURE THEATRE H**

SYMPOSIUM
Nanotechnology and imaging

Chairs:
Gang Li (Belfast, UK)
Andrew McCaskie (Newcastle)

08.30 IS11 SKELETAL TISSUES AT THE NANOSCALE
Laurent Bozec (London, UK)

Oral Communications

Chairs:
Andrew McCaskie (Newcastle)
John Fisher (Leeds, UK)

09:00 OC28 IMAGING OF THE MUSCULOSKELETAL SYSTEM USING 3D ULTRASOUND
E Ross[1], TJ MacGillivray[2], H Simpson[1], WN McDicken[3]*
[1]Edinburgh Orthopaedic Engineering Centre, University of Edinburgh, Edinburgh Royal Infirmary; [2]Wellcome Trust Clinical Research Facility, Western General Hospital, EH4 XU; [3]Medical Physics, University of Edinburgh, Edinburgh Royal Infirmary

09:10 OC29 A NEW UNDERSTANDING OF STRUCTURAL ADAPTATION OF TENDON TO MECHANICAL LOAD
AP Rumian[1], ERC Draper[1,2,3], AL Wallace[1], AE Goodship[2,3]*
[1]Imperial College, London, UK; [2]Veterinary Basic Sciences, Royal Veterinary College, Hatfield, UK; [3]Institute of Orthopaedics and Musculoskeletal Sciences, UCL, London, UK

09:20 OC30 A NEW METHOD TO MEASURE THE INTER-FRACTURE SITE MOVEMENTS (IFMS) DYNAMICALLY BY MEANS OF STEWART PLATFORM ALGORITHM
F Li[1], JH Kuiper[2], SA Khan[3], C Hutchinson[1,3], CE Evans[1]*
[1]University of Manchester; [2]The Robert Jones & Agnes Hunt Orthopaedic Hospital; [3]Hope Hospital, Manchester

09:30 OC31 EXPRESSION PROFILING OF MATRIX METALLOPROTEINASES IN DUPUYTREN'S DISEASE
P Johnston[1,2], AJ Chojnowski[1], RK Davidson[2], GP Riley[3], ST Donell[1], IM Clark[2]*
[1]Norfolk & Norwich University Hospital, Norwich; [2]School of Biological Sciences, University of East Anglia, Norwich; [3]Rheumatology Research Unit, Addenbrooke's Hospital, Cambridge

09:40 OC32 THE MOLECULAR AND CELLULAR RESPONSE OF HUMAN BONE TO MECHANICAL STIMULATION
V Mann[1], G Kogianni[1], C Huber[1], A Voultsiadou[1], AHRW Simpson[1], DB Jones[2], BS Noble[1]*
[1]Musculoskeletal Tissue Engineering Collaboration (MTEC), University of Edinburgh Medical School; [2]Dept. Experimental Orthopaedics and Biomechanics, Philipps University Marburg

09:50 OC33 SINGLE INJECTION OF THROMBIN-RELATED PEPTIDE (TP508) IN A SLOW-RELEASING PREPARATION ENHANCED BONE CONSOLIDATION DURING DISTRACTION OSTEOGENESIS
G Li[1], C Wan[1], H Wang[2], DH Carney[2], JT Ryaby[2]*
[1]Musculoskeletal Education and Research Unit, Queen's University Belfast, Musgrave Park Hospital, Belfast, BT9 7JB, UK; [2]Research Department, OrthoLogic, 1275 West Washington Street, Tempe, USA

10:00 OC34 BIOMATERIAL SURFACE ARCHITECTURE DICTATES CELL:CELL INTERACTIONS AND REGULATES OSTEOBLAST DIFFERENTIATION
G Kirmizidis, MA Birch*
University of Newcastle, Newcastle upon Tyne, UK

10:10 OC35 AN INNOVATIVE EX VIVO MODEL FOR CHONDROGENESIS AND OSTEOGENESIS
JC Pound, DW Green, HI Roach, ROC Oreffo.*
Bone & Joint Research Group, Developmental Origins of Health and Disease, University of Southampton, Southampton, SO16 6YD, UK

10:20 OC36 AUGMENTATION OF ALLOGRAFT WITH HUMAN BONE MARROW STROMAL CELLS: VALIDATION OF CELL SURVIVAL, PROLIFERATION, OSTEOGENIC PHENOTYPE AND MECHANICAL STRENGTH
BJ Bolland, K Partridge, AMR New, DG Dunlop, ROC Oreffo*
Southampton, UK

10.30 GARDEN END OF GARDEN COURT

COFFEE and poster viewing

11.20 NUFFIELD THEATRE

Oral Posters

Chairs:
Brendon Noble (Edinburgh, UK)
Marian Young (Bethesda, USA)

11:20 OP1 GENE, PROTEIN AND ELECTROPHYSIOLOGICAL EXPRESSION OF ION CHANNELS ON HUMAN TENOCYTES
M Magra[1,2], S Hughes[1], A ElHaj[1], N Maffulli[1,2]*
[1]Institute of Science and Technology, Keele University, Staffordshire, UK; [2]Department of Trauma and Orthopaedic Surgery, University Hospital of North Staffordshire, UK

11:25 OP2 BONE MARROW-DERIVED MESENCHYMAL STEM CELLS CAN FUSE WITH TUMOUR CELLS IN VITRO AND HOME TO TUMOURS IN VIVO
J Xiang, C Song+, G Li+, D Hirst**
School of Pharmacy and +Musculoskeletal Research Unit, School of Biomedical Sciences, Queen's University Belfast, Musgrave Park Hospital, Belfast, BT9 7JB, UK

11:30 OP3 THE SIGNIFICANCE OF WIRE TENSION IN FINE-WIRE FIXATORS: AN ANALYSIS OF THE EFFECT OF WIRE TENSION ON THE STRESS DISTRIBUTION AT THE WIRE-BONE INTERFACE
F Alvi[1], L Yang[2], T N Board[3]*
[1]North West Deanery; [2]Division of Clinical Sciences (North), University of Sheffield; [3]University of Manchester

11:35 OP4 ADENOSINE MODULATES MESENCHYMAL STEM CELL DIFFERENTIATION INTO OSTEOBLASTS
BAJ Evans[1], C Elford[1], J Ham[2]*
[1]Department of Child Health[2]Centre for Endocrine and Diabetes Sciences, School of Medicine, Cardiff University, Cardiff, UK

11:40 OP5 A NOVEL APPROACH TO CREATING A BIO MIMETIC HYDROXYAPATITE COATING ON FINE SURFACE FEATURES TO ENHANCE OSSEOINTEGRATION OF EXTERNAL FIXATION SCREWS
A Unnithan, R Wells, G Blunn, A Goodship*
RNOH, Stanmore

11:45 OP6 MUTATIONS IN THE RANK SIGNAL PEPTIDE ALTER THE SUBCELLULAR LOCALISATION OF RANK AND PREVENT LIGAND-DEPENDENT ACTIVATION OF NF KAPPA BETA
JC Crockett[1], A Duthie[1], J Greenhorn[1], DI Scott[1], SH Ralston[2], MH Helfrich[1], MJ Rogers[1]*
[1]University of Aberdeen, Aberdeen, UK; [2]University of Edinburgh, Edinburgh, UK

11:50 OP7 BIOMIMETIC APPROACHES TOWARDS THE DESIGN AND SYNTHESIS OF TISSUE ENGINEERING SCAFFOLDS
DW Green, J Pound, K Partridge, R Tare, D Walsh, S Mann, R Oreffo*
Southampton, UK

11:55 OP8 FUNCTIONAL AND ULTRASTRUCTURAL STUDIES OF OSTEOCLASTS FROM OSTEOPETROTIC PATIENTS
F Coxon[1], K Mackenzie[2], J Greenhorn[1], L Van Wesenbeeck[3], A Frattinni[4], A Pangrazio[4], A Villa[4], P Odgren[5], M Helfrich[1]*

[1]Department of Medicine and Therapeutics, Institute of Medical Sciences, University of Aberdeen; [2]Histology- EM core facility, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK; [3]Department of Medical Genetics, University and University Hospital Antwerp, Antwerp, Belgium; [4]Instituto di Tecnologie Biomediche Avanzate, Consiglio Nazionale delle Ricerche, Segrate, Milano, Italy; [5]Department of Cell Biology, University of Massachusetts Medical School, Worcester MA01655, United States

12:00 OP9 CHANGES IN THE CHARACTERISTICS OF ARTICULAR CHONDROCYTES DURING OSTEOARTHRITIS
A Aziz, HI Roach*

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

12:05 OP10 OSTEOBLASTS RESPOND TO MILD HEAT STRESS BY A CHANGE IN OSTEOPROTEGERIN (OPG) AND RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPA B LIGAND (RANKL) RATIO
S Meghji[1], ST Ong[1,2], A Maddi[1,3], G Vinayahan[1]*

[1]UCL Eastman Dental Institute, London, UK; [2]Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia; [3]Faculty of Dentistry, National University of Singapore, Singapore

12:10 OP11 EXPERIMENTALLY VALIDATED FINITE ELEMENT MODEL OF A HUMAN TIBIA WITH A UNICOMPARTMENTAL KNEE REPLACEMENT
HA Gray[1], AB Zavatsky[1], L Cristofolini[2,3], HS Gill[4]*

[1]Dept of Engineering Science and [4]Dept of Orthopaedic Surgery (OOEC), University of Oxford, UK; [2]Medical Technology Lab, Rizzoli Orthopaedic Institutes, Bologna, Italy; [3]Engineering Faculty, University of Bologna, Italy

12:15 OP12 LEFT-HANDEDNESS IN WOMEN IS ASSOCIATED WITH A LOWER RISK OF FRACTURE BEFORE THE AGE OF 40
J Finigan[1], JA Clowes[2], NFA Peel[1], R Eastell[1]*

[1]University of Sheffield, Sheffield, UK; [2]Mayo Clinic School of Medicine, Rochester, MN, USA

12.30 NUFFIELD THEATRE

BRS AGM and awards

12.30 GARDEN END OF GARDEN COURT

LUNCH and poster viewing

13.30 NUFFIELD THEATRE

SYMPOSIUM
Clinical Disorders - Paediatric/ Metabolic bone disease

Chairs:
Jonathan Tobias (Bristol, UK)
Brigitte Scammell (Nottingham, UK)

13.30 IS12 BONE FRAGILITY IN CHILDREN
Nick Bishop (Sheffield, UK)

14.00 IS13 THE LINKS BETWEEN BODY COMPOSITION AND BONE
Ian Reid (Auckland, New Zealand)

14.30 IS14 FUTURE MANAGEMENT OF FRACTURE RISK
David Marsh (London, UK)

15.00 GARDEN END OF GARDEN COURT

TEA

15.30 NUFFIELD THEATRE

Clinical Workshop

Chairs:
Mark Cooper (Birmingham, UK)
Chris Edwards (Southampton, UK)

15.30 IS15 NICE GUIDELINES
Richard Keen (London, UK)

15.50 IS16 RISK ASSESSMENT
John Kanis (Sheffield, UK)

16.10 IS17 OSTEOPOROSIS TREATMENT IN THE BIOLOGICS ERA
Richard Eastell (Sheffield, UK)

16.30 IS18 VITAMIN D: WHO NEEDS SUPPLEMENTATION AND WITH WHAT?
Frazer Anderson (Southampton, UK)

15.30 LECTURE THEATRE J

SYMPOSIUM
Musculoskeletal regeneration

Chairs:
Chris Evans (Boston, USA)
Tony Miles (Bath, UK)

15.30 IS19 THERAPEUTIC CELL THERAPY AND TISSUE ENGINEERING: NOVEL MAGNETIC STATEGIES FOR GROWTH CONTROL AND DELIVERY
Alicia El-Haj (Keele, UK)

Oral Communications

Chairs:
Chris Evans (Boston, USA)
Tony Miles (Bath, UK)

16:00 OC43 ENHANCED CHONDROGENESIS OF INFRAPATELLAR FAT PAD STEM CELLS IN HYPOXIC CONDITIONS
W Khan, A Adesida, JG Andrew, TE Hardingham*

United Kingdom Centre for Tissue Engineering, University of Manchester

16:10 OC44 DYNAMIC RELEASE OF GROWTH FACTORS FROM FRESH FROZEN CANCELLOUS BONE ALLOGRAFT

TN Board[1,2], P Rooney[2], PR Kay[1]*

[1]Wrightington Hospital, Wigan, UK;[2]Tissue Services R&D, National Blood Service, Liverpool, UK

16:20 OC45 THE EFFECT OF LOCAL ADMINISTRATION OF PHENYTOIN ON FRACTURE HEALING: AN EXPERIMENTAL STUDY

M Mathew, R Sen, R Nada*

Postgraduate Institute of Medical Education and Research, Chandigarh, India

16:30 OC46 SFA(PATH); A MODIFICATION OF THE SFA GRADING SYSTEM OF CHONDROPATHY IN OSTEOARTHRITIS FOR USE WITH PATHOLOGICAL SAMPLES

*A Yousef *[1], R Hill[1], D Wilson[1], DA Walsh[1,2]*

[1]Sherwood Forest Hospitals NHS Trust, Mansfield Road, Sutton-in-Ashfield, NG17 4JL, UK; [2]Academic Rheumatology, University of Nottingham Clinical Science Building, City Hospital, Hucknall Road, Nottingham, NG5 1PB, UK

16:40 OC47 THE SAGITTAL PLANE KINEMATICS OF A NEW UNICOMPARTMENTAL KNEE REPLACEMENT: A CADAVERIC STUDY OF THE 'DOMED' OXFORD LATERAL UNICOMPARTMENTAL KNEE REPLACEMENT

JA Gallagher[1], C Lee[2], M Schablowski[2], P Aldinger[2], H Gill[1], DW Murray[1]*

[1]Nuffield Orthopaedic Centre, University of Oxford, UK; [2]Heidelberg Orthopaedic Clinic, University of Heidelberg, Germany

16:50 OC48 NINE YEARS FOLLOW UP AFTER THE FIRST AUTOLOGOUS HUMAN DISC REGENERATIONS AND REPLANTATIONS

BE Gerber[1], M Biedermann[2]*

[1]University Hospital Lewisham, London, UK; [2]Hopital de La Chaux-de-Fonds, Switzerland

Invited Speaker Abstracts

IS1
EMBRYONIC STEM CELLS

Stephen Minger
Wolfson Centre for Age-Related Diseases, King's College London, UK

There has been significant interest in the therapeutic and scientific potential of human embryonic stem (ES) cells since they were first isolated in 1998. If human ES cells could be differentiated into suitable cell types, stem cells might be used in cell replacement therapies for degenerative diseases such as Type I diabetes and Parkinson's disease, or to repopulate the heart following myocardial damage. However, there is a significant shortage of high quality human ES cell lines and few research groups have experience in the propagation and manipulation of these cells. It is thus essential for the development of human stem cell technology, and the larger goal of cellular replacement therapy for human disease, that additional human cell lines are generated.

We are addressing this important issue using the combined expertise of the Stem Cell Biology Laboratory and the Assisted Conception Unit at King's College London. With local ethical approval and under licence from the UK Human Fertilisation and Embryology Authority, we have been establishing high quality human ES cell lines from a novel source of human embryos. To date, we have derived three human ES cell lines, including one that encodes the most common genetic mutation resulting in Cystic Fibrosis. In addition, much of our work is focused on the generation of human ES cell-derived, therapeutically important cell populations including neural, retinal, pancreatic, cardiac and endothelial stem cells. The tightly regulated yet permissive environment in the UK for human stem cell research, coupled with the government's commitment to the establishment of a centralised stem cell bank offers the UK the opportunity to be a leading player in the field of human regenerative medicine.

IS2
GENE THERAPIES IN MUSCULOSKELETAL DISEASE

Chris Evans
Harvard Medical School, Boston, MA, USA

Depending upon how you view the field, there are four main areas where gene therapy can be usefully applied to treat orthopaedic conditions (JAAOS 13: 230-242, 2005): Mendelian diseases, chronic complex diseases, cancer and tissue repair. Of these, a substantial body of literature exists only for chronic diseases, especially arthritis, and tissue repair. Arthritis is the most advanced of these applications, and there have already been four phase I clinical trials of gene therapy in subjects with rheumatoid arthritis. These have provided preliminary evidence of safety and efficacy. Animal models of osteoarthritis also respond to gene transfer and the first clinical trial in this area is under development.

Among the challenges for a successful gene therapy of a chronic condition such as arthritis is the need for prolonged periods of transgene expression. This is difficult and limits the choice of possible vectors for gene delivery. Tissue repair is an attractive additional target for gene therapy because a transgene will only need to be expressed for a short period of time-possibly just a few weeks. Moreover, the level of transgene expression may not need to be regulated very closely. Existing technology can already provide the necessary duration and level of transgene expression very effectively. Pre-clinical studies have demonstrated impressive healing of osseous lesions in response to the transfer of osteogenic genes. Gene-based approaches to cartilage repair are also a popular subject of research. Preliminary experiments have been conducted for improving the repair of meniscus, ligaments and tendon by gene transfer.

Collectively, the pre-clinical data are very encouraging and suggest an eventual clinical use of such technologies in certain settings. Among the issues that will determine the clinical utility of gene therapy in the orthopaedic context are safety, cost and feasibility.

IS3
GENETICS OF OSTEOARTHRITIS

John Loughlin
University of Oxford, Institute of Musculoskeletal Sciences, Botnar Research Centre, UK

Epidemiological studies, including twin-pair analyses and relative-risk studies, support a major genetic component to osteoarthritis (OA) susceptibility. OA is not however a Mendelian trait but instead falls in to the complex, multifactorial class of diseases. It has gradually become apparent that the nature of the OA genetic risk is likely to vary between different skeletal sites and may also vary between the sexes, although this latter observation is based on a small number of studies and needs further investigation to confirm it's veracity.

With a genetic component established the next step was a hunt for the risk alleles. So far there have been four genome-wide linkage scans and two large-scale, gene-based SNP association studies performed on OA relative-pairs and case-control cohorts, respectively. The linkage scans revealed a number of highly significant loci, some of which have started to yield their susceptibility loci. The association studies have also identified a number of interesting hits. So far, robust data implicating the following genes has been generated: 1) the secreted frizzled-related protein 3 gene FRZB on chromosome 2q32.1 in a UK population; 2) the asporin gene ASPN on chromosome 9q22.31 in a Japanese population; 3) the calmodulin 1 gene CALM1 on chromosome 14q32.11, also in a Japanese population and; 4) the leucine-rich repeat protein gene LRCH1 on chromosome 13q14 in a UK and a Newfoundland population.

These recent findings suggest that OA genetic risk is acting principally on chondrocyte differentiation, proliferation and the general homeostatic balance of the cartilage extracellular matrix rather than through structural defects in the matrix. This is an important observation since signalling pathways are modifiable. The new genetics has therefore identified targets for new drug development as well as loci that can now be genotyped to identify at-risk individuals for more focussed clinical trials.

IS4
DNA METHYLATION AND THE SILENCING OF GENE EXPRESSION

John Newell-Price
Senior Lecturer in Endocrinology, University of Sheffield

DNA methylation is associated with silencing of gene expression. The predominant mechanism involves methylation of DNA and subsequent recruitment of binding proteins that preferentially recognize methylated DNA. In turn, these proteins associate with histone deacetylase and chromatin remodelling complexes to cause stabilization of condensed inactive chromatin. The opposite may also hold: targeting of methylation might depend on altered (repressed) chromatin structure.

To assess the impact of these mechanisms on gene expression we have been studying the Proopiomelanocortin gene (POMC) as a model system. POMC plays an essential role in the regulation of the hypothalamo-pituitary-adrenal axis, adrenal development and obesity. The peptide product, POMC, is cleaved to a variety of peptides including adrenocorticotrophin (ACTH), which in turn stimulates adrenal steroidogenesis, especially glucocorticoids, which in turn feedback to inhibit POMC expression. Excess glucocorticoid exposure, either endogenously produced or from therapeutic use, leads to osteoporosis, muscle atrophy, diabetes mellitus and hypertension – Cushing's Syndrome.

In ACTH-dependent Cushing's syndrome POMC is over-expressed. The highly tissue-specific 5' promoter of human POMC is activated in corticotroph adenomas of the pituitary and rarely may also be activated non-pituitary sites. The factors involved in transcription in the corticotrophs of the anterior pituitary gland are well delineated, but the mechanism of activation in non-pituitary sites is not fully understood. This promoter is embedded within a defined CpG island, and, in contrast to somatically expressed CpG island promoters reported to date, is methylated in normal non-expressing tissues, but is specifically unmethylated in expressing tissues, tumours and the POMC-expressing DMS-79 small-cell lung cancer cell line. Low-level DNA methylation in vitro is sufficient for silencing of expression. Active demethylation does not appear to occur, implying that methylation and expression patterns are likely to be set early in neoplastic transformation, and that targeted de novo methylation might be a potential therapeutic strategy. To address whether a repressed gene is targeted for de novo methylation we have also assessed the effect of long-term suppression of POMC expression by glucocorticoids.

IS5
WEAR OF TOTAL JOINT REPLACEMENTS

John Fisher
University of Leeds, Leeds

Over two million total replacement joints are implanted in patients every year, and ten percent of the population of developed countries benefit from a joint replacement in their life times. Historically joint replacements were undertaken in the elderly, and with a conservative life style (1 million steps per year) and life expectancy of 10 to 20 years, tribological demands of between 10 to 20 million cycles were expected.

In the modern age, younger more active patients are demanding joint replacements. They have life expectancy of 20 to 40 years and an increased activity of up to 5 million steps per year means a ten fold increase in lifetime tribological demand of 100 to 200 million cycles. Wear and osteolysis is a common cause of failure in joint replacement, and increased tribological demands now requires high performance bearings in both hip and knee replacements. Additionally patients are demanding improved biomechanical function such as range of motion which in the hip requires larger diameter femoral heads in hip replacements.

Conventional metal or polyethylene bearing for hip replacements have been improved with the introduction of highly cross linked polyethylene which lead to a four fold reduction in wear. Unfortunately the debris released for highly cross linked polyethylene is smaller and more reactive and only results in a two fold reduction in functional osteolytic potential.

Alumina ceramic on ceramic bearings have substantially reduced wear and osteolytic potential compared to highly crossed linked polyethylene, and are available in head sizes 28 to 36 mm.

Metal on metal bearings also have substantially lower wear than highly cross linked polyethylene and have the added advantage of being available in head sizes to 60 mm diameter. Additionally as a lubrication sensitive bearing the wear has been shown to decrease as the head size increases, again addressing both tribological performance and biomechanical function.

Research studies and the resulting development of prosthesis, have shown that a combination of biomechanical design and biomaterial combination can start to address the ten fold tribological demand of the young and active patient.

IS6
OSTEOCYTE MODIFICATION OF EXTRACELLULAR MATRIX

Lynda Bonewald
School of Dentistry, University of Missouri-Kansas City, USA

It is thought that the osteoblast initiates and controls all processes associated with and necessary for the production and mineralization of bone. It has been proposed that the osteoblast leaves behind within its trailing osteoid all proteins and organelles sufficient to initiate and propagate mineralization in a timely fashion and that the embedding osteoid-osteocyte becomes a static, inactive cell when it looses the majority of its cytoplasm, thereby simply existing for years or decades as a place holder in bone. Once surrounded by mineral, considerable data supports the function of a mature osteocyte as a mechanotranslating, signaling cell. However, data is starting to emerge that the osteoid-osteocyte may play a role in the mineralization process and that the mature osteocyte can modify its local environment.

As the osteoid-osteocyte embeds, it appears to generate mineralized, spherical structures of 50-200nM as the cytoplasm shrinks and dendritic processes extend. These structures dislodge from the cell surface and associate with collagen fibrils where they increase in size and coalesce to form mineralized bone. Proteins highly expressed in the embedding cell and the mature osteocyte such as E11, Dentin Matrix Protein 1, PHEX and MEPE are most likely playing a role in this process. Even once the cell is embedded in mineralized matrix, it has the capacity to influence its microenvironment. For example, the number of canaliculi appear to increase with age. Another example is the response of osteocytes to agents such as steroids. Glucocorticoid not only induces osteocyte apoptosis, but the remaining viable (though compromised) osteocytes appear to have the capacity to enlarge their lacunae and leach mineral from the surrounding matrix. Capturing these changes has been difficult because osteocyte modification of their microenvironment takes weeks, months, or even years in contrast to the very rapid action of osteoclasts, hours and days, and the rapid action of osteoblasts, days and weeks. Therefore, the mature osteocyte may be a rapid responder with regards to mechanotransduction, but requires extended periods of time to modify its microenvironment.

IS7
SMALL LEUCINE-RICH PROTEOGLYCANS IN THE SKELETON

Marian F Young
Craniofacial and Skeletal Diseases Branch, NIDCR, NIH, Bethesda, MD 20892, USA

Small Leucine-Rich Proteoglyans (SLRPs) are major skeletal ECM components that comprise a family of 13 members containing repeats of a leucine-rich motif. To examine SLRP function, we generated mice deficient in one or more member and analyzed them at the tissue, cell and molecular levels. Mice deficient in biglycan (a class I SRLP) acquired early onset osteopenia due to a decreased ability to make new bone. Experiments using normal and biglycan deficient calvaria cells showed that biglycan controls BMP binding and activation. To attain a comprehensive picture of downstream effectors controlled by the presence of biglycan, microarray analyses was performed using mRNA from biglycan-deficient osteoblasts treated with or without BMP. Numerous differentially regulated mRNA's were identified related to cell cycle, differentiation and apoptosis. New molecular circuits potentially connecting biglycan to osteoblast function were uncovered and are currently under investigation. The observed defects in biglycan-deficient osteoblasts led us to speculate that biglycan could also modulate osteoclast function. Osteoblast-osteoclast co-culture and induced osteolysis experiments using normal and biglycan-deficient mice confirmed this theory. The SLRP decorin is closely related to biglycan and is up-regulated in the context of biglcyan deficiency. To test whether there is redundancy/compensation in SLRP function, we made mice deficient in biglycan and decorin. These mice displayed a more profound osteopenia compared to mice deficient in only one of the SLRPs. To examine the molecular mechanisms that caused this osteopenia we cultured osteogenic bone marrow cells from the doubly deficient mice. We found these cells proliferate faster than normal cells but, unlike the singly deficient biglycan, they were not defective in differentiation. Moreover we found a "hypersensitivity" to TGF-beta that eventually led to premature apoptosis. This premature cell death appeared to deplete osteogenic precursors, and is the likely cellular basis for decreased osteogenesis in this animal model. To test compensation in distantly related SLRPs mice deficient in both biglycan (a class I SLRP) and fibromodulin (a class II SLRP) were made. They acquired early onset osteoarthritis caused by weak tendons that ossified prematurely. We plan to use these mouse models to identify early molecular events causing skeletal abnormalities dependent on SLRP function. This research was supported by the IRP-DIR, NIH.

IS8
WHAT IS INTERVERTEBRAL DISC DEGENERATION, AND HOW DOES IT AFFECT ADJACENT VERTEBRAE?

Michael A Adams
Department of Anatomy, University of Bristol, Southwell Street, Bristol, BS2 8EJ, UK

The aims of this review are to a) distinguish intervertebral disc degeneration from growth, ageing, and adaptive remodelling, and b) to explain how disc degeneration alters the loading applied to vertebral bodies in such a manner that they becomes vulnerable to injury.

Proposed definitions: 1) the process of disc degeneration is an aberrant, cell-mediated response to progressive structural failure; 2) a degenerate disc is one with structural failure combined with accelerated or advanced signs of ageing; 3) early degenerative changes should refer to accelerated age-related changes in a structurally intact disc; 4) degenerative disc disease should be applied to a degenerate disc which is also painful.

Structural disruption plays a central role in these definitions because structural defects such as endplate fracture, radial fissures and herniation are easily-detected, unambiguous markers of impaired disc function. They are not inevitable with age, and are more closely related to pain than any other feature of ageing discs. Structural failure is irreversible, because adult discs have limited healing

potential. It also progresses, by physical and biological mechanisms, and so is a suitable marker for a degenerative process. Biological progression occurs because structural failure uncouples the local mechanical environment of disc cells from the overall loading of the disc, so that disc cell responses become inappropriate or 'aberrant'. Animal models confirm that cell-mediated changes always follow structural failure due to trauma. This definition of disc degeneration simplifies the issue of causality: excessive mechanical loading disrupts a disc's structure and precipitates a cascade of cell-mediated responses leading to further disruption. Underlying causes of disc degeneration include genetic inheritance, age, inadequate metabolite transport, and loading history, all of which can weaken discs to such an extent that structural failure occurs during the activities of daily living.

Degenerated discs press unevenly on the vertebral bodies, concentrating stress anteriorly when the spine is flexed, and posteriorly when the spine is extended. Furthermore, in the usual upright postures, disc narrowing transfers up to 80% of compressive loading on to the neural arch. Consequently, anterior regions of the vertebral body become weakened and prone to wedge fractures.

IS9
DEVELOPMENTAL ORIGINS OF OSTEOPOROTIC FRACTURE

Cyrus Cooper
Professor of Rheumatology and Director, MRC Epidemiology Resource Centre, University of Southampton, UK

Osteoporosis is a skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. The cumulative incidence of fracture from age 50 years is estimated at around 50% among white women and 20% among white men. Preventive strategies against osteoporotic fracture can be targeted throughout the life course. Thus, modification of physical activity and dietary calcium/vitamin D nutrition in the elderly and during midlife, should complement high risk approaches entailing appropriate measurement of bone mineral density and targeting of anti-resorptive and formation stimulating drugs. Prevention of osteoporotic fracture can also be directed earlier in the life course. Environmental influences during early life interact with the genome in establishing the functional level of a variety of metabolic processes which are involved in the pathogenesis of osteoporotic fracture. The evidence that osteoporosis risk might be programmed in this way stems from four groups of studies: (1) Epidemiological studies which confirm that subjects who are born light and whose growth falters in the first year of postnatal life, have significantly lower bone size and mineral content, at age 60 to 75 years; (2) Epidemiological cohort studies have demonstrated that subsequent lower trajectories of childhood growth are associated with an increased risk of hip fracture among such men and women; (3) Detailed physiological studies of candidate endocrine systems which might be programmed have shown that birthweight and growth in infancy alter the functional settings of the GH/IGF-1, and hypothalamic pituitary adrenal axes; (4) Studies characterising the nutrition, body build and lifestyle of pregnant women which relate these to the bone mass of their newborn offspring, have identified a number of important determinants of reduced fetal mineral accrual (maternal smoking, low maternal fat stores and maternal vitamin D deficiency, intense levels of weight-bearing physical activity in late pregnancy). Follow-up studies of randomised controlled trials of vitamin D supplementation in infancy suggest persisting benefits in adolescence and young adulthood. These data suggest that undernutrition and other adverse influences arising in fetal life or immediately after birth have a permanent effect on body structure, physiology and metabolism, which might independently influence the later risk of cardiovascular disease and osteoporotic fracture.

IS10
DETERMINANTS OF BONE GROWTH IN CHILDHOOD: INSIGHTS FROM THE ALSPAC COHORT

Jonathan Tobias
University of Bristol

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a unique population-based birth cohort, recruited from approximately 14,000 pregnant women between 1991-2. We have used this study to investigate determinants of bone development in childhood, based on total body DXA scans performed in 7333 and 7006 children at age 9.9 and 11.8 years respectively, using a Lunar Prodigy. This presentation will focus on recent findings which suggest a hitherto unrecognised influence of adiposity on periosteal bone growth, based on our results for height-adjusted total body bone area. Initially, we examined how social position affects bone development, by linking measures of social position such as level of maternal education, ascertained by questionnaire completed during pregnancy, to age 9.9 DXA data. Higher social position was found to be inversely associated with height-adjusted total body bone area. On the other hand, higher social position was positively associated with bone area after adjusting for weight. These findings suggest that social position exerts opposing effects on bone growth, such that higher social position increases bone size through effects on longitudinal growth, whereas lower social position enhances periosteal growth through effects on weight.

This suggestion that weight is an important determinant of periosteal growth led to further studies which highlight the role of adiposity in bone development. For example, we found that fat mass mediates the effect of weight on the relationship described above between social position and bone area. In addition, our subsequent studies revealed that fat mass as measured by DXA at age 9.9 years is an important positive determinant both of bone area at age 9.9, and of percentage increase in bone area over the following two years, even after adjustment for height and lean mass. We then examined whether effects of physical activity on fat mass modify those on the skeleton. An inverse relationship was observed between height-adjusted bone area, and moderate and vigorous physical activity (MVPA) as assessed by accelerometer recordings, in 4457 age 11 children. However, MVPA was positively related to bone area after adjusting for both height and fat mass. These findings suggest that although MVPA stimulates periosteal bone growth, this action is opposed by the tendency of MVPA to reduce fat mass. Taken together, our findings suggest that adipose tissue represents an important influence on bone development in childhood, though the mechanisms involved remain to be elucidated.

IS11
SKELETAL TISSUES AT THE NANOSCALE

Abstract not received

IS12
BONE FRAGILITY IN CHILDHOOD

Nick Bishop
University of Sheffield and Sheffield Children's Hospital

If bone fragility is reflected in a high risk of fracture, then it would appear that as a group children have bones that are as fragile as those of the elderly. Fractures are common in childhood; around one third of children will fracture at least once by age 17 years and the peak age-specific incidence of fractures in boys of 3% and 1.5% in girls is surpassed only by that of women over 85 years and not at all in men.

Fractures are commoner at all ages from 2-17 in boys. For boys and girls, fracture incidence peaks around the time of maximal height velocity. Children who fracture appear to have reduced bone mass for body size compared with those who do not fracture. Cohort studies suggest an increased risk of fracture in children receiving inhaled therapy for asthma, but this has not been confirmed in case control studies. Recurrent fractures occur in approximately 6% of children. Recurrent fractures are more common in children who are overweight and physically less active, in those who avoid milk and in those who consume excessive quantities of carbonated drinks.

There are a number of disorders that predispose to fractures with low bone mass in infancy and childhood; these include osteogenesis imperfecta, idiopathic juvenile osteoporosis and metabolic bone disease of prematurity. Great progress has been made in the treatment of these disorders over the last 10 years, particularly with the introduction of bisphosphonate therapy as part of multidisciplinary management for the osteoporotic disorders. The diagnosis of bone fragility in infancy and its differentiation from child abuse remains a contentious issue.

IS13
THE LINKS BETWEEN BODY COMPOSITION AND BONE

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Body weight impacts on both bone turnover and bone density, and is therefore an important risk factor for vertebral and hip fractures, ranking in importance alongside that of age. The effect of body weight is probably contributed to by both fat mass and lean mass, though in postmenopausal women, fat mass has been more consistently demonstrated to be important. A number of mechanisms for the fat-bone relationship exist and include the effect of soft tissue mass on skeletal loading, the association of fat mass with the secretion of bone active hormones from the pancreatic beta cell (including insulin, amylin, and preptin), and the secretion of bone active hormones (eg, estrogens and leptin) from the adipocyte. These factors alone probably do not fully explain the observed clinical associations, and further study of the actions on bone of novel hormones related to nutrition is an important area of further research. An understanding of this aspect of bone biology may open the way for new treatments of osteoporosis. More immediately, the role of weight maintenance in the prevention of osteoporosis is an important public health message that needs to be more widely appreciated.

IS14
FUTURE MANAGEMENT OF FRACTURE RISK

Abstract not received

IS15
NICE GUIDELINES

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In the UK, the National Institute for Health and Clinical Excellence (NICE) is an independent organisation responsible for providing national guidance on the promotion of good health and the prevention and treatment of ill health. NICE publishes guidance which aims to ensure that promotion of good health and patient care in the NHS are in line with the best available evidence of clinical effectiveness and cost effectiveness. NICE guidance falls into 3 categories: Interventional Procedures, Technology Appraisals and Clinical Guidelines.

With relevance to osteoporosis, guidance on the interventional procedures vertebroplasty and kyphoplasty was initially published in September 2003, with revised recommendations published in April 2006.[1,2] Both these techniques are now approved for use in patients with painful vertebral fractures refractory to more conservative treatment. These procedures should only be undertaken with prior discussion by a specialist multidisciplinary team that includes a radiologist and a spinal surgeon, and when there are facilities for good imaging, and arrangements for good access to a spinal surgery service. Clinicians should also have received training to reach an appropriate level of expertise before carrying out these procedures.

The technology appraisal on treatments for established postmenopausal osteoporosis was published in January 2005.[3] This guidance reviewed the evidence for bisphosphonates (alendronate, etidronate and risedronate), raloxifene and teriparatide. Interventional thresholds for treatment were defined on the basis of age, BMD and clinical risk factors. This guidance is now being updated to include strontium ranelate, although it will not review the evidence for other drugs that are now available such as ibandronate. The Appraisal Consultation Document (ACD) on this area will be published in late July 2006. In addition, a further ACD will be published at this time on the treatment of primary osteoporosis. This presentation will critically review some of the key data and assumptions that are being included in the NICE models, and the potential impact this guidance will have on clinical practice.

The development of clinical guidelines will assist in the management of osteoporosis other than postmenopausal disease (i.e. secondary disease, osteoporosis in men, paediatric osteoporosis). It is hoped that these guidelines will also include information similar to the WHO risk algorithm to assist in calculating an individual patient's 10-year risk of fractures. Recommendations for follow-up and monitoring will also be useful for clinical practice. To date, no draft of these guidelines has been published.

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IS16
RISK ASSESSMENT

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At present, treatment is largely directed on the basis of bone mineral density (BMD). In the UK, treatment is recommended when the T-score for BMD is found to be less than -2.5 SD. The same T-score has, however, quite a different significance at different ages. For example, the 10-year probability of hip fracture for women in the UK with a T-score of -3 SD is 3.2% at the age of 50 years, but 19.8% at the age of 80 years. Thus, fracture risk prediction is optimised by integrating information on risk factors that contribute to fracture risk independently of BMD. A major programme of the WHO Collaborating Centre at Sheffield has been to identify and validate readily used risk factors.

Risk factors for fractures have been identified from 12 prospective population-based cohorts comprising 250,000 person-years of observation with 3,500 osteoporotic fractures. Clinical risk factors that contribute to fracture risk independently of BMD include age, previous fragility fractures, a family history of fracture, rheumatoid arthritis, smoking, exercise, alcohol and the use of oral glucocorticoids. Their combined use with (or without) BMD enhances the sensitivity of fracture prediction without sacrificing specificity. The utility of the risk factors has been validated in the independent population-based cohorts of 230,000 individuals followed for 1.2 million person-years.

The ability to assess fracture risk from clinical risk factors permits intervention in men and women that is based not solely on BMD. Therefore, diagnostic thresholds for osteoporosis (based on BMD) differ from intervention thresholds. Because of the many techniques available for fracture risk assessment, the ten year probability of fracture is the desirable parameter to determine intervention thresholds. The setting of intervention thresholds is ultimately dependent on health economic considerations. When BMD is used as a test alone, an intervention threshold of –2.5 SD is cost-effective. In the presence of other independent risk factors less stringent criteria are appropriate so that intervention can be directed to individuals where hip fracture probability ranges from 2% to 10% (depending on age). These thresholds, derived from Sweden or the UK, require modification in different countries to take account of different costs and risks that vary markedly in different regions of the world.

IS17
OSTEOPOROSIS TREATMENT IN THE BIOLOGICS ERA

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We have several treatments licensed for osteoporosis, or in the clinical development phase. Some of these have been developed as a result of our better understanding of bone biology. The two that I will discuss are the antibody to RANK-L (denosumab) and the 1 to 34 fragment of parathyroid hormone (teriparatide). The RANK-L pathway is critical in the control of bone resorption, and a number of approaches have been developed to inhibit it, including the use of OPG, RANK-fc, and RANK-L antibodies. The last of these is now in Phase III clinical trials and has been shown in postmenopausal women to have effects on bone turnover markers and bone mineral density that are at least as great as the bisphosphonate alendronate. The onset and offset of these effects are rapid. The potency of this agent on bone resorption may make it effective in other disorders of

high bone remodelling. Teriparatide is the 1 to 34 N-terminal fragment of parathyroid hormone and is licensed for the treatment of severe osteoporosis. It is given by daily subcutaneous injection. It results in a large and early increase in bone formation markers and bone histomorphometry had shown that this reflects the production of de novo bone formation on quiescent trabecular surfaces as well as periosteal new bone formation. The resulting large increases in bone mineral density have been associated with a reduction in the rates of vertebral and non-vertebral fractures. There are questions that remain, 1) can it be given by other routes; 2) which patients benefit most; 3) how should it be used with anti-catabolic therapy (before, during or after) and should multiple courses be given?

IS18
VITAMIN D – WHO NEEDS SUPPLEMENTATION AND WITH WHAT?

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Vitamin D (calciferol) is not really a vitamin at all, as it is synthesised endogenously in sunlight-exposed skin. Calciferol is a steroid hormone precursor which in its activated form, calcitriol, regulates calcium uptake from the gut and renal calcium handling. Deficiency depletes the skeletal calcium reservoir, causing osteomalacia, rickets and osteoporosis. Deficiency also impairs neuromuscular co-ordination, influencing falls risk. Additionally, calcitriol mediates some aspects of cell growth and differentiation, with putative roles in autoimmunity, allergy and carcinogenesis.

Recommended dietary intake levels of vitamin D are difficult to establish. It is highly unusual for any endocrine pathway to depend on external supply of the pre-hormone, which suggests that skin synthesis is the more physiological source.

Calciferol is sequestered in fat and calcitriol synthesis is tightly controlled by regulation of hydroxylation in the kidney. Vitamin D status is therefore assessed by measurement of its circulating intermediate metabolite, 25-hydroxy-calciferol (25-OH-D). Levels below about 20nmol/l are clearly associated with osteomalacia and are described as “deficiency”, but calcium homeostasis remains abnormal at 25-OH-D levels up to around 50-70nmol/l. This suboptimal range is referred to as “insufficiency” and there is considerable controversy over where to set its upper limit, with US authorities favouring higher limits than most others. These higher target levels are effectively unachievable through diet alone.

Vitamin D insufficiency is very common in older people, and supplementation is widely advocated for the prevention of bone disease. It is available as either vitamin D3 (colecalciferol), identical to the molecule made in human skin, or vitamin D2 (ergocalciferol), produced by irradiation of fungi. D2, which is cheaper to manufacture, is effective for the prevention of osteomalacia/rickets, but there is growing evidence that it is much less potent than D3 in maintaining optimum calcium balance.

Clinical trials of vitamin D supplements for fracture prevention have shown conflicting results and there is no consensus on their interpretation. Current thinking in the UK favours offering combined vitamin D3 (700-800iu) and calcium supplements to housebound older people and care home residents for primary fracture prevention, and as an adjunct to anti-osteoporosis drugs such as bisphosphonates for secondary prevention.

IS19
THERAPEUTIC CELL THERAPY AND TISSUE ENGINEERING: NOVEL MAGNETIC STATEGIES FOR GROWTH CONTROL AND DELIVERY

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New therapies involving cells have enormous potential for applications in regenerative medicine and treatment of disease - the challenge lies in controlling the behaviour and activity of these cells in vitro and in vivo. Enabling technologies are being developed which allow us to grow and condition cells in vitro. These technologies facilitate the growth of tissues in a 3D environment and enable physiologically relevant cues such as mechanical strain to be delivered in vitro and ultimately in vivo. In addition, new technologies are being developed which allow delivery of cells in a patient and maintenance of these cells at the site of repair. In our lab, we are investigating the use of magnetic strategies to manipulate and control differentiation of cells in a bioreactor environment or remotely in vivo in the patient. In this presentation, we report our recent work on tagging specific mechanosensitve receptors with magnetic nanoparticles which results in downstream cell signalling and gene activation using time varying remote magnetic fields. This technique is applicable to monolayer cultures and cell seeded 3D constructs for tissue engineering of bone and cartilage. By controlling the mechanosesnors directly on the cells within the construct, we are no longer reliant on using slow degrading materials which are capable of withstanding significant load bearing for bone tissue engineering thus enhancing rapid turnover and construction of biological matrices in vitro. Using a magnetic force bioreator developed in our lab, we describe our new strategies for internalisation of magnetic nanoparticles which can bind to key receptor sites on the internal membrane. A comparison of receptor sites tagged such as integrins and ion channels will be described with resultant effects on bone cell signalling and formation. Recent work has investigated conditioning of MSCs (Poetics, Ltd) in monolayer culture demonstrating an upregulation of bone cell markers such as osterix, CBFA1 and osteopontin after 1 week of cyclical loading in culture. The magnetic nanoparticle technology has the potential to be applied directly in vivo in animals models and ultimately in clinical treatments. We describe recent investigation into localisation of stem cells in vivo using magnetic tagging.

OC1

CLONAL PLASTICITY AND IN VIVO BONE FORMATION OF HUMAN FETAL FEMUR-DERIVED CELLS

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Strategies to augment bone repair remains a major unmet clinical issue. To address this need we have examined the potential of human fetal-derived mesenchymal tissue, as a robust and facile model system for elucidation of stromal lineage differentiation and development of musculoskeletal regeneration strategies. Cells were derived from human fetal femurs collected at 8-12 weeks post-conception and grown from explants or via enzyme digestion with collagenase. Cultures were compared to unselected and immunoselected mesenchymal stem cells. Single cells, obtained by FACS, were expanded as clonal populations under basal conditions before application of differentiation media. Fetal cells were also seeded onto poly-lactic acid/hydroxyapatite (PLA/HA) scaffolds and maintained for 28 days in vitro as well as analysis in vivo using a subcutaneous implant model in SCID mice. Molecular characterisation using quantitative PCR demonstrated increased expression of BMP receptor IA and the stromal antigen CD166 in fetal-derived cells in comparison to unselected and selected (STRO-1) adult mesenchymal populations. Fetal-derived cells expressed the stromal antigen STRO-1, which was maintained in basal culture over 14 days, as demonstrated by immunofluorescence. Differentiating populations of fetal cells expanded from a single cell, in osteogenic conditions, demonstrated expression of type I collagen, alkaline phosphatase and osteopontin genes in basal and osteogenic conditions. Peroxisome proliferation-activated receptor-gamma gene expression was only detected under adipogenic conditions. Osteogenic growth factors ascorbate, dexamethasone and BMP-2 increased alkaline phosphatase specific activity in fetal-derived cells. On biomimetic PLA/HA scaffolds, fetal cells displayed extensive accumulation of matrix both in vitro and in vivo, following implantation in SCID mice, as demonstrated by organized collagenous matrix production detected by Sirius red staining and birefringence polarized microscopy. Confirmation of in vivo human fetal femur cell-derived tissue formation was confirmed using anti-human vimentin antibody. Clonal plasticity along adipogenic and osteogenic lineages and chondrogenic pellet cultures was demonstrated using single fetal cells. These studies demonstrate the multipotential properties of fetal-derived cells, indicating their stem cell nature in direct comparison with adult-derived mesenchymal cells. These cells offer a unique comparative model for fundamental research with potential in skeletal regeneration.

OC2

SURVIVAL OF XENOGENEIC BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN A XENO-TRANSPLANTATION MODEL

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Mesenchymal stem cells (MSCs) are immunosuppressive and have been used to facilitate tissue repair in the context of allogeneic implantation. However, xenogeneic cell transplantation has not been fully explored. The present study investigated the feasibility of xenogeneic MSCs implantation in mice.

MSCs were harvested from the bone marrow of GFP rats (Green Fluorescent Protein transgenic rats), and cultured as previously described. 1 million GFP MSCs were loaded onto the synthetic HA/TCP porous Skelite blocks and implanted intramuscularly into the quadriceps of the MF1 and SCID mice. After 11 weeks, the implants were harvested and processed for histology examination. Upon termination, the mononuclear cells from the peripheral blood of each animal were also collected for mixed lymphocyte culture to examine lymphocyte proliferation potential and T-cell mediated cell lysis (cytotoxic) assays.

In the SCID mice, there was sparse osteoid tissue formation in the implants, whereas only dense connective tissues were seen in the implants of the MF1 mice. Osteocalcin mRNA expression was confirmed in the osteoid tissues in the implants from the SCID mice, but it was not detected in the MF1 mice by RT in situ PCR examination. Cells of GFP-rat origin were observed in both the MF1 and SCID mice (more so in the SCID mice) after 11 weeks implantation, which were confirmed by positive immunostaining of anti-GFP antibody. In the MF1 mice after 11 weeks xenogeneic MSCs implantation, the rate of lymphocyte proliferation was significantly increased when mixed with the GFP-MSCs compared to that of mixed lymphocyte culture assays in the SCID or MF1 mice without xenogeneic MSCs implantation, suggesting that implantation of xenogeneic MSCs has promoted host anti-graft immunogenic responses towards to otherwise immunosuppressive MSCs.

In conclusion, xenogeneic rat MSCs transplanted in immunocompetent mice has survived for prolonged period, but their function was comprised to certain extent and this may be due to the increased host anti-graft immune sensitization after exposed to the xenogeneic MSCs.

OC3

FUNCTIONAL CHOLINERGIC SIGNALLING MECHANISMS AND THE CENTRAL REGULATION OF HUMAN MESENCHYMAL STEM CELLS

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The anti-osteogenic effects relayed by leptin and mediated by osteoblastic adrenoceptors have resulted in a greater appreciation of the fundamental role of the central nervous system (CNS) in the regulation of bone mass. In many tissues receiving neuronal innervation both cholinergic and adrenergic signalling mechanisms co-exist and operate to functionally antagonise the activity of one another. Expression of adrenoceptors by osteoblasts and their progenitors raised the intriguing possibility that cholinergic signalling mechanisms may be present and play a role in the central regulation of bone mass by regulating the activity of bone cells. In this study we aimed to address whether acetylcholine (ACh) signalling mechanisms were present and functional in human mesenchymal stem cells (hMSCs). By RT-PCR and flow cytometry we detected expression of the nicotinic ACh receptor subunits alpha3, 5 and 7, and the muscarinic receptor 2 by hMSCs. Significantly, we demonstrated that hMSCs also express the key enzyme involved in ACh synthesis, (choline acetyltransferase), synthesise and secrete ACh and express acetycholinesterase (AChE), which is responsible for terminating cholinergic activity. Using confocal calcium imaging we demonstrated that stimulation of hMSCs with the non-specific cholinergic agonist carbachol or the specific agonists nicotine or muscarine induced immediate and transient increases in intracellular calcium concentration. Similar effects were observed in response to AChE inhibition, providing evidence of receptor function and autocrine ACh signalling mediated by local ACh secretion and receptor activation. Analysis of intracellular signalling revealed that exposure of hMSCs to carbachol, muscarine or AChE inhibitor attenuated the production of cAMP and stimulated the MAPK signalling pathway via phosphorylation of ERK. Furthermore, using a luciferase reporter gene containing four copies of the consensus AP1 transcription factor binding site, we demonstrated that activation of ACh signalling significantly increases AP1-mediated gene transcription. Taken together these data indicate that functional cholinergic signalling mechanisms exist in hMSCs, and provides evidence that ACh plays a novel role in the local autocrine regulation of hMSC function. Endogenously, this pathway may contribute to the central regulation of bone mass and its manipulation may provide new therapeutic avenues for the treatment of bone disorders caused by aberrant bone mass homeostasis.

OC4

CAN MESENCHYMAL STEM CELLS PLAY A ROLE IN INCREASING NEW BONE FORMATION?

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Introduction: The current practice of impaction allograft to fill large defects in revision total hip replacements is sometimes useful but clinical results are inconsistent. Other studies have shown that addition of mesenchymal stem cells (MSC) in blocks of hydroxyapatite (HA) scaffold can enhance new bone formation in a critical sized defect. However, no study has been conducted on combined MSCs with morselised allograft and HA granules. It is

hypothesized that impaction of allograft or HA granules seeded with MSCs or osteoprogenitors will enhance new bone formation compared with the groups without MSCs.

Materials and Methods: Six sheep were used for the study. Each sheep received 8 scaffolds which were embedded in both paraspinal muscles. Groups were: 1) 3.5g allograft, 2) 3.5g allograft with MSCs, 3) 3.5g allograft with osteoblasts; 4) 3.5g of 50:50 allograft/HA, 5) 3.5g of 50:50 allograft/HA with MSCs, 6) 3.5g of 50:50 allograft/HA with osteoblasts; 7) a block of HA, 8) a block of HA with MSCs. The experimental scaffolds were seeded with either 10x106 MSCs/ml or 10x106 MSC-derived osteoprogenitors/ml, in 3ml autologous plasma. Grafts were impacted twenty times at 3KN. At eight weeks, samples were sectioned for histology analysis. Areas of new bone formation were measured as percentage to total available spaces. ANOVA was used for statistical analysis.

Results: Addition of MSCs increased new bone formation in allograft (4.98%), allograft/HA (5.15%) and HA block (7.09%) compared with their controls at 2.24%, 1.96% and 1.96% respectively. Statistical study showed significant increase in 50:50 allograft/HA with MSCs compared with 50:50 allograft/HA only (p=0.046) and 50:50 allograft/HA with osteoprogenitors (p=0.028). No difference was found in allograft groups. For the HA block groups, addition of MSCs showed a significant new bone increase compared to the control (p=0.028).

Conclusion: Addition of MSCs to the allograft and HA granules will enhance new bone formation after impaction which can be used for revision total hip replacements, especially when allograft and HA is mixed. However, addition of osteoprogenitors has not achieved the similar results. This study encourages a further clinical investigation of impaction tissue-engineered graft to repair bone defects in revision total joint replacements.

OC5

EXCESSIVE LOCAL GLUCOCORTICOID GENERATION MAY EXPLAIN THE IDIOPATHIC OSTEOPOROSIS OF GLYCOGEN STORAGE DISEASE TYPE 1A

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Glycogen storage disease type 1a (GSD1a, von Gierke's disease) is a mild inborn error of metabolism characterised by fasting hypoglycaemia. It is due to loss of function mutations in the glucose-6-phosphatase (G6Pase) gene. The G6Pase enzyme is located in the endoplasmic reticulum (ER), converts glucose-6-phosphate (G6P) to glucose and is critical for gluconeogenesis. An unexplained feature of GSD1a is severe osteoporosis associated with high bone resorption and low bone formation. We hypothesised that osteoporosis was due to increased glucocorticoid generation in osteoblasts due to changes in enzyme cofactor levels within the ER lumen. 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) is a bidirectional enzyme that interconverts cortisone (inactive) and cortisol (active). Glucocorticoid activation requires cofactor generation by another ER enzyme hexose-6-phosphate dehydrogenase (H6PDH), a process that also requires G6P. Increased G6P levels in GSD1a might thus indirectly stimulate glucocorticoid activation. We explored this hypothesis in patients with GSD1a and in wild type and knockout H6PDH mice.

Urinary corticosteroid metabolite excretion was examined in 3 patients with GSD1a. All patients had high indices of global 11beta-HSD1 activity (cortisone to cortisol conversion)(1.86+/-0.36, mean+/-SEM compared to 1.15+/-0.25 in 60 healthy controls; p<0.01). Total corticosteroid generation was not increased. When challenged with oral cortisone patients had an exaggerated generation of cortisol (AUC=218.96µmol/L/240mins) compared to controls (AUC=74.49+/-4.24µmol/L/240mins). These data were supported by in vitro 11beta-HSD1 assays performed in triplicate on mouse liver microsomes in the presence and absence of a G6Pase inhibitor (10nM sodium vanadate). Steroid activation was significantly increased in the presence of vanadate (18.2+/-0.1% versus 8.1+/-1.0% (control); p<0.01) an effect not seen in H6PDH knockout mice. Although primarily a gluconeogenesis enzyme G6Pase was expressed at high levels in human osteoblasts as was 11beta-HSD1 and H6PDH. Deletion of H6PDH reversed the directionality of 11beta-HSD1 in mouse osteoblasts.

In patients with GSD1a local glucocorticoid generation is substantially increased in tissues that express 11beta-HSD1. Although we cannot exclude an impact of other metabolic factors on the bone disease in this condition, increased osteoblastic glucocorticoid generation is likely to make an important contribution.

OC6

THE DETECTION OF HUMAN EMBRYONIC AND ADULT STEM CELL SURVIVAL IN IMMUNE DEFICIENT AND IMMUNE COMPETENT RAT MODELS OF BONE REPAIR

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We have used human Embryonic Stem cells (hESC) and human Mesenchymal Stem Cells (hMSC) in rat models of bone repair in order to assess the efficacy of these cells for treatments of trauma and skeletal diseases. Graft survival is considered to be of key importance to efficacy of these treatments. Therefore the aim of this study was to develop a technique for identifying implanted cells in histological preparations without the need for genetic engineering of the implanted cells.

Methods: In our experiments hES and hMSC were pre-differentiated during cell culture towards the osteoblast lineage, and then implanted in a Demineralised Bone Matrix (DBM) carrier into an experimentally created full thickness calvarial bone lesion. The animals were sampled seven days and fourteen days after implantation into either immune deficient (RNU-Foxn1rnu) or immune competent (wild type) Sprague Dawley rats. Fluorescent In Situ Hybridisation (FISH) using whole human genome probes identified the human cells within the host lesion site.

Results: Our results have demonstrated that hESC and hMSC derived cells survive in both immune competent (wild type) and immune compromised (nude) animals for the initial seven days post implantation. On the other hand while both the hESC and hMSC derived cells are capable of surviving for at least 14 days in immune compromised animals they do not survive for this period of time in immune competent animals.

Discussion: It appears that the cell/DBM graft is not rejected within seven days even when exposed to the wild type hosts T cell response. However longer term survival required an immune deficient model that is lacking in a T cell response. This data points to interesting future studies regarding which components of the host response are responsible for xenogenic stem cell implant rejection.

OC7

MILD PRIMARY HYPERPARATHYROIDISM IS ASSOCIATED WITH LOW BMD BUT NOT FRACTURE RISK, QUALITY OF LIFE OR MORTALITY IN COMMUNITY DWELLING ELDERLY WOMEN

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Conservative management of mild primary hyperparathyroidism is recommended in patients without a history of complications. We wished to examine the impact of mild hyperparathyroidism (serum calcium <0.4mmol above the upper reference range) on fracture risk, mortality and quality of life in elderly community-dweeeling women.

Albumin-adjusted serum calcium and serum phosphate were measured in 5212 women aged at least 75 years at entry to the MRC Hip Fracture Study. Serum PTH was measured if adjusted serum calcium was greater than 2.60mmol/l to confirm the diagnosis as women with non-parathyroid hypercalcaemia were excluded from the study. Other assessments included measurements of total hip BMD (Hologic QDR4500), quality of life (Euroqol), incident fractures and mortality.

The mean age in the population was 79.5±3.8 years at entry to the study. A total of 126 (2.4%) women fulfilled the above criteria for primary hyperparathyroidism (cases). There were no significant differences in mean age, height or weight compared to the rest of the population (controls). Quality of life as measured by Euroqol was similar in cases and controls (P=0.75). Serum phosphate and serum creatinine were significantly lower (mean difference -0.12±0.01mmol/l) or higher (+3.7±1.7micromol/l) respectively in the cases. Bone mineral densities at the total hip (0.70±0.14 vs.

0.76±0.14g/cm2, P<0.001) and all hip sub-regions (P<0.003) were significantly lower in mild primary hyperparathyroidism. There was no significant interaction between the presence of mild primary hyperparathyroidism and the ability of clodronate to prevent fractures (20% reduction). Over a median follow-up of 4 years, the incidence of all clinical fractures was similar in the cases compared to controls (17.5 vs. 14.3%, OR 1.27, 95%CI 0.80-2.02, P=0.31) though the trend was stronger for peripheral fractures (OR 1.53, 0.83-2.80). The incidence of hip fracture was similar in both groups (3.2 vs. 3.6% in controls, OR 0.88, 0.32-2.42, P=0.81). The mortality rate in the cases was slightly but not significantly lower than in the controls (12.7 vs. 14.5%, OR 0.86, 0.50-1.45, P=0.7).

Mild primary hyperparathyroidism appears to have no major consequences for elderly women living in the community. Conservative management appears to be an appropriate management strategy.

OC8

ATDC5: AN IDEAL CELL LINE FOR STUDYING CHONDROCYTE DIFFERENTIATION AND MODELLING CARTILAGE FORMATION

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Cartilage is a realistic target for tissue engineering given the avascular nature and cellular composition of the tissue. Much of the work in this field has been largely empirical, indicating the need for alternative approaches to the design of cartilage formation protocols. Given the heterogeneity associated with human mesenchymal populations, continuous cell lines may offer an alternative to model and simplify cartilage generation protocols. We therefore exploited the potential of the murine chondrocytic ATDC5 cell line to, i) delineate the process of chondrocyte differentiation in monolayer culture and three-dimensional micromass pellet culture systems, and ii) model cartilage formation utilising appropriate scaffold and bioreactor (perfused and rotating) technologies. Monolayer cultures of ATDC5 cells over a 28-day period in presence of insulin demonstrated various stages of chondrocyte differentiation- proliferative, pre-hypertrophic, hypertrophic and finally, mineralisation of cartilaginous nodules. This was confirmed by gene and protein expression, by qPCR and Western blotting respectively, of chondrogenic differentiation markers- Sox-9, Bcl-2, Type II and X collagens. Pellet cultures of ATDC5 cells under chondrogenic conditions (10 ng/ml TGF-beta3, 1X ITS {insulin, transferrin, selenium}, 10 nanomolar dexamethasone, 100 micromolar ascorbate-2-phosphate) illustrated a gradual progression from an aggregation of cells at day 7, to initiation of matrix synthesis at day 14, followed by formation of well-defined cartilaginous structures at day 21. Chondrogenic differentiation at day 21 was evident by numerous proliferative/ pre-hypertrophic chondrocytes, staining for Sox-9, Aggrecan, Type II collagen and PCNA, lodged in distinct lacunae embedded in cartilaginous matrix of proteoglycans and Type II collagen. Inclusion of TGF-beta3 in the chondrogenic medium during pellet culture beyond 21 days maintained the pre-hypertrophic phenotype, even at day 28. In contrast, removal of TGF-beta3, addition of 50 nanomolar thyroxine and reduction of dexamethasone to 1 nanomolar in the chondrogenic medium stimulated hypertrophy at day 28, evident by down-regulation of Sox-9 expression. ATDC5 cells cultured on Polyglycolic acid fleece in the rotating bioreactor or encapsulated in chitosan /alginate and cultured in the perfused bioreactor for 21 days, formed cartilaginous explants reminiscent of hyaline cartilage. Thus, ATDC5 cells constitute an ideal cell line to elucidate the steps of chondrocyte differentiation and cartilage formation.

OC9

ZOLEDRONATE REDUCES EARLY ACUTE BONE LOSS AT THE HIP FOLLOWING SPINAL CORD INJURY

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There is currently limited data on strategies to prevent bone loss and reduce future fracture risk in patients with spinal cord injury (SCI). This prospective, randomised, open label study was designed to assess the role for zoledronate in acute SCI.

Patients aged 18 yrs or over with acute SCI (complete or incomplete), who were within 3 months of injury were invited to participate. Active treatment was a single infusion of 4mg zoledronate. All patients received standard care. Bone mineral density (BMD) at the lumbar spine and hip was measured at baseline, 3, 6 and 12 months using DXA (Hologic QDR-Delphi).

In total, 16 patients (7 controls and 9 zoledronate) were studied. The groups were well matched for baseline characteristics (age, sex distribution, baseline BMD and duration since injury). The mean age of the patients was 32.4 yrs (range 18 to 57). 5 of the 9 actively treated patients experienced a transient, acute phase reaction, but no long-term complications were observed.

Significant bone loss was apparent in the control group at the hip by 3 months. This loss continued over the 12 month study period, with overall changes of -15.9% at the total hip, -14.6% at the femoral neck, and -18.2% at the trochanter. Treatment with zoledronate prevented bone loss at the total hip and trochanteric sites, with between group differences of 12.5% and 11.0% respectively (p< 0.05). A similar trend was also seen at the femoral neck, although by 12 months the between group difference was only 4.3% (NS). No significant change in lumbar spine BMD was seen in the control group over the study period. Zoledronate treatment was associated with a significant increase in BMD (+5.8%) from baseline, but the between group difference was not significant.

These data confirm that hip BMD is rapidly lost following SCI, with loss commencing as early as 3 months and continuing up to 12 months. A single treatment with zoledronate 4 mg appears effective in preventing this bone loss, although longer term studies > 1 yr duration will be required to determine the optimal management strategy in these patients.

OC10

A FINITE ELEMENT STUDY OF A HIP RESURFACING ARTHROPLASTY WITH AND WITHOUT A STEM

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Hip resurfacing arthroplasty (HRA) is increasingly carried out as an alternative to total hip arthroplasty (THA) in young patients. During the procedure, a metal stem on the retrosurface of the HRA is inserted into the femoral head to ensure the implant is located centrally with respect to the femoral neck. It has been suggested that the stem may interfere with bone loading. In light of this, the current study employed finite element (FE) models to investigate the change in the HRA-implanted bone mechanics as a result of removing the stem. FE models of a cadaveric femur pre- and post-HRA surgery were analysed to determine changes in bone stress/strain.

The implanted models simulated geometry for a cemented HRA with and without a non-cemented stem (HRA-Stem and HRA-NoStem, respectively) and included more accurate multiple material parameters to simulate the non-homogeneous material distribution in the femoral bone. The models included loading conditions simulating an instant at 10% of the gait cycle. Bone stresses/strains in the femoral head and neck of the implanted models were compared with the intact condition to assess the change in bone mechanics. Changes in cement mantle stresses between the HRA-Stem and HRA-NoStem models were also compared.

When comparing similar volumes of bone in the femoral neck, both HRA models showed a similar variation in stress from the intact condition and bone stresses were low in comparison to the ultimate strength of cortical bone. There was less change in peak strain energy in the femoral head of the HRA-NoStem model than the HRA-Stem model. Cement mantle stresses in the HRA-NoStem model were slightly higher than for the HRA-Stem model and the peak compressive stress was close to the fatigue limit for bone cement.

These preliminary results suggest that the bone loading is more normal without the stem. However, there are increased cement mantle stresses.

OC11

CHROMOSOMAL ABERRATIONS IN PATIENTS WHO HAVE HAD METAL-ON-METAL HIP BEARINGS IN-SITU FOR IN EXCESS OF 30 YEARS

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Background: Metal-on-Metal (MoM) hip bearings are being implanted in ever increasing numbers and into ever-younger patients. The consequence of chronic exposure to metal ions is a cause for concern. Therefore, by using cytogenetic biomarkers, we investigated a group of patients who have had MoM bearings in-situ for in excess of 30 years.

Method: Whole blood specimens were obtained from an historical group of patients who have had MoM bearings in-situ for in excess of 30 years. Blood was also obtained from an age and sex matched control group and from patients with Metal-on-Polyethylene (MoP) components of the same era.

The whole blood was cultured with Pb-Max karyotyping medium and harvested for cytogenetics after 72 h. The 24 colour FISH (Fluorescent In Situ Hybridisation) chromosome painting technique was performed on the freshly prepared slides allowing chromosomal mapping. Each slide was evaluated for chromosomal aberrations (deletions, fragments and translocations) against the normal 46 (22 pairs and two sex) chromosomes. At least 20 metaphases per sample were scored and the number of Aberrations per cell calculated.

Results: Chromosomal aberrations, including deletions, fragments and translocations were only detected in the peripheral blood lymphocytes isolated from the group that had MoM bearings. These changes were not present in the age and sex matched control group. The chromosomal aberrations were also detected in the patients previously exposed to MoM bearings who have been revised to a MoP articulation.

Conclusion: We have detected dramatic chromosomal aberrations in peripheral blood lymphocytes in a group of patients chronically exposed (over 30 years) to elevated metal ions. It is not known whether these aberrations have clinical consequences or whether they are reproduced in other cells in the body. The results emphasise the need for further investigations into the effect of chronic exposure to elevated metal ions produced by Orthopaedic implants.

OC12

THE FRICTION BEHAVIOUR DURING THE WEAR OF METAL ON METAL HIP JOINTS WITH DIFFERENCE CLEARANCE

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The clearance between the femoral head and the acetabular cup can significantly affect the lubrication, the wear and the lifetime of metal on metal (MOM) hip joints. The objective of this study was to compare the frictional behaviour of MOM joints with different clearance.

Two CoCrMo MOM 50mm diameter hip joints, with a small diametral clearance of 17 microns and a big diametral clearance of 212 microns, were used in this study. The friction measurement was carried on the wear patches of MOM bearings during a long-term wear simulator test. A dynamic trapezoidal-form loading cycle was applied to the femoral head with a minimum load of 100N during the swing phase and a maximum load of 2000N throughout the stance phase. A simple harmonic motion of amplitude +/-24 degree was applied to the femoral head in the flexion-extension plane with a frequency of 1 Hz. The friction torque was measured at 0, 0.8, 1.3, 1.9, 4 and 5.5 million cycles using 6 different viscosities of 25% new born calf serum.

The results show that the friction factors (f) of small clearance were generally higher than those of big clearance and this difference became wider with the progress of wear. The lower f of big clearance, especially in the lower range of Sommerfeld number (z) after 5.5 million cycles, is significant and will affect the ultimate performance of prostheses as this range has closer rheological properties to synovial fluid and represents long term wear conditions. At the same time, the friction factors were always higher every time when measured from high z to low z, although this difference became slightly smaller with the progress of wear, which indicates that there is still direct contact between the bearings. The lower friction factor when increasing z, is due to the wear and bedding-in with the progress of the measurement. It is concluded that large clearance has lower friction factor than small clearance, and full fluid film lubrication is unlikely to have developed between the MOM bearings in this study, even with a small clearance and high viscosity.

OC13

LONG-BONE REGENERATION USING GUIDING MEMBRANES: CRYOPRESERVED ARTERIAL ALLOGRAFTS VERSUS SYNTHETIC MEMBRANES

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Background and objective: In guided tissue regeneration a membrane is used for defect isolation to protect it against invasion from surrounding tissues and to keep intrinsic healing factors 'in situ'. This technique has been successfully used in maxillo-facial surgery, but short experience has been reported in long-bone defects, with synthetic membranes and with variable results. In the other hand, calcification and ossification inside the arterial wall have been described. The aim of the study was to evaluate the use of cryopreserved aorta allografts as membranes for guided tissue regeneration in comparison with expanded poly-tetra-fluoro-ethylene (e-PTFE) synthetic membranes.

Methods: Prospective, randomized, blinded study in 15 New-Zeland rabbits. 10 mm mid-diaphyseal defects were created in both radii: 10 defects were covered with a cryopreserved aortic allograft as a tube, 10 with an e-PTFE membrane and 10, with no barrier membrane, served as controls. Animals sacrifice at 6-12-24-30 months. Studies: X-rays, CT, MR, morpho-densitometric analysis, electronic and optical microscopy. Immuno-cytochemistry on tissues and arterial wall cells cultured.

Results: None of the control defects healed. Nine defects covered with an artery completely reconstituted, but only six of those covered with e-PTFE, with a nearly normal cortical-medullar pattern and with progressive increasing in density and thickness of medullar and cortical to values similar to those of the normal bone. Histological studies showed no inflammatory response to the arterial graft, direct union between the artery and the regenerated bone and even mature bone between the elastic laminae of the arterial wall, suggesting superior biocompatibility properties. Immuno-cytochemistry and ultrastructural studies suggest that arterial allografts could act not only as membrane barriers, with additional osteoinductive properties due to trans-differentiation of viable arterial wall cells (endothelial, smooth muscle and/or tissue specific stem cells) towards osteoblastic cells, and also due to ossification secondary to changes in proteins of the arterial extracellular matrix. This could be the application of the process of arterial wall calcification and ossification (usually seen in arteriosclerosis, gender, diabetes or kidney failure) for regeneration of long-bone defects.

Conclusion: Cryopreserved aortic allografts can be used as membrane barriers for guided bone regeneration, with superior results to e-PTFE membranes.

OC14

ESTIMATE OF THE FUNCTIONAL IMPROVEMENT IN THUMB CMC JOINT OSTEOARTHRITIS AFTER A SINGLE INTRA-ARTICULAR STEROID INJECTION

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We present a unique prospective study which estimates the median sustained stage related improvement in pain and hand function predicting symptomatic relief period with high accuracy with a single steroid injection.

Patients were grouped into stages, I to IV according to the Eaton and Glickel radiological criteria. The steroid injection contained 40mg triamcelone and 1% lidocaine. The response was assessed by DASH and a visual analogue score before and at six-week interval. We used the Kaplan-Meier method to estimate median length of sustained improvement by grade of disease, with 95% confidence interval. All the patients were injected by an upperlimb physiotherapist (DD). Post injection review was carried out by an independent observer(MK).

Forty patients were studied: 33 females and 7 males. The age ranged from 53 to 81 years, (mean 65years). No patient was lost to follow-up. Mean duration of symptoms were 36 months. Six patients has stage I disease(15%), eighteen patients had stage II disease (45%), ten patients had stage III disease (25%) and six patients has stage IV disease (15%). Pain score ranged from 4 to 9 on visual analogue score. Reduction in pain visual analogue score was noticed in all but 3 patients. With the exception of Grade III patients, DASH scores decreased significantly at 6 weeks (Grade I 14.9, Grade II 19.3, Grade III 6.2 and Grade IV 10.0.). With the exception of Grade IV patients, pain scores decreased significantly at 6 weeks. In Grade II patients, over half had sustained symptomatic relief at 6 months. So on average, we can expect grade I patients to sustain symptomatic relief for an average of 17 weeks. The true average is likely to be between 13 and 21 weeks. For grade II patients, most will still have improved at 6 months. Grade III and IV patients have an identical prognosis of 4 weeks, though the true prognosis may be between 2 and 6 weeks.

In conclusion it is possible to predict the period of symptomatic improvement in each of the four disease stages. This allows the treating clinician to discuss the outcome of treatment with reasonable accuracy.

OC15

DETECTION OF MATRIX METALLOPROTEINASES IN PRIMARY FROZEN SHOULDERS

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In patients with DM (Diabetes Mellitus types I & II), primary frozen shoulders tend to be refractory to all forms of treatment. We collected tissue from the joint capsule of shoulder joints from a variety of patients undergoing surgery as follows:

I - Diabetic Group (DFS): patients with DM who have primary frozen shoulders.

II - Other patients suffering from primary frozen shoulders (FS)

III- Control group (NS). Patients undergoing shoulder surgery that does not involve stiffness of the gleno-humeral joint.

Tissue was collected from near to the rotator interval under arthroscopic control. Fibroblast lines were established by serial passage. Thereafter they were exposed to graded concentrations of insulin in vitro for 24 hours and the supernatant retained for assay. Fibroblast lines were analysed from 3 subjects in each group (n=9). Luminex multiplex analysis was performed for MMPs (Matrix Metalloproteinases). TIMP-1 (Tissue Inhibitor of MetalloProteinases) expression. Informed consent was obtained from all subjects.

Results: Production of MMP 1,2,3 and 8 by fibroblast lines were distinct between patient groups. MMP-1 production in DFS (mean 716pg/mL) was significantly reduced compared to FS derived patient cells (mean 972pg/mL) ($p=0.0138$, Mann-Whitney Test). Moreover, striking differences were observed when fibroblasts from DFS patients were compared with those from NS controls (mean 5898pg/mL) ($p<0.000$). Calculating MMP-1/TIMP-1 ratios revealed significantly lower ratios in DFS (2597), or FS (2860) compared with NS (24,326) ($p <0.001$). There was no significant difference between ratios of MMP1/TIMP1 in DFS and FS ($p=0.977$). MMPs 7,9,12 and 13 were not detected in any of the samples.

This is the first time these enzymes have been measured and quantified in cells derived from shoulder tissues. Primary Frozen Shoulders produce less MMPs and have a smaller MMP/TIMP ratio than controls. Similarly the diabetic patient derived cells produce less MMP-1, at an even lower level. These deficiencies in MMP1 production may reflect an altered capacity for local tissue re-modelling. MMP modulation may allow therapeutic intervention in the diabetic and frozen shoulder group of patients.

OC16

FUNCTIONAL IONOTROPIC GLUTAMATE RECEPTORS IN HUMAN FIBROBLAST-LIKE SYNOVIOCYTES MODULATE IL-6 AND MMP-2 EXPRESSION

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Rheumatoid arthritis (RA) is a systemic autoimmune disease affecting 350,000 people in the UK. Within synovial joints, synoviocytes form a destructive pannus that degrades articular cartilage and bone. Synovial fluid glutamate levels increase 54 fold in RA patients and are also elevated in animal models of inflammatory and osteoarthritis. To determine whether elevated glutamate levels contribute to RA pathology we investigated which synovial joint tissues express glutamate receptors and whether glutamate stimulation influences synoviocyte phenotype.

Various glutamate receptor mRNAs (NMDAR1, KA1, AMPAGluR2, AMPA GluR3, mGluR4) were expressed in tissues of the rat knee. All receptors were expressed in the patella. The fibrocartilagenous meniscus and articular cartilage chondrocytes expressed mGluR4 and both AMPA receptor subunits. Human synoviocytes expressed NMDAR1 and KA1 mRNA.

To determine whether glutamate receptors were functional in human synoviocytes, cells were preloaded with a fluorescent indicator of intracellular calcium (iCa 2+) and stimulated with glutamate or specific agonists (NMDA or kainate, 500mM). Glutamate stimulated release of iCa2+ in 25% of synoviocytes whereas NMDA and Kainate each stimulated 15% of cells. NMDA responses increased to 57% in the absence of Mg2+ consistent with the inhibitory effect of Mg2+ on this receptor.

To determine whether activation of glutamate receptors can influence human synoviocyte phenotype, we cultured synoviocytes in various glutamate concentrations (50mM to 2mM) and measured effects of glutamate receptor antagonists on release of a proinflammatory cytokine (IL-6) and degradative enzymes (MMP2 and 9). In some RA patients, glutamate stimulation increased synoviocyte pro MMP-2 release. TIMP1 and TIMP2 release were not affected by glutamate stimulation or co-treatment with receptor antagonists.

IL-6 expression varied greatly in human synoviocytes derived from different RA patients (0-120pg/ml media). However, the AMPA/KA receptor antagonist NBQX significantly reduced IL-6 release at all glutamate concentrations. This inhibition was greater than that by CFM2 (AMPAR antagonist), indicating that activation of kainate receptors in human synoviocytes may induce IL-6 release.

We conclude that glutamate receptors are functional in human synoviocytes and regulate release of MMP-2 and IL-6 Thus glutamatergic signalling may contribute to RA pathology and represent a new therapeutic target.

OC17

SEVERE MECHANICAL LOADING CAN CAUSE CERVICAL INTERVERTEBRAL DISCS TO PROLAPSE

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Introduction: The cervical spine can be severely loaded in bending during sporting injuries and 'whiplash'. Compressive loading could also be high if some advanced warning of impact stimulated vigorous ('protective') contraction of the neck muscles. Combined bending and compression can cause some lumbar discs to herniate in-vitro (1) but the outcome depends on spinal level, and may not be applicable to cervical discs. We test the hypotheses: a) that cervical discs can prolapse in-vitro, and b) that prolapse leads to irregular stress distributions inside the disc.

Material and methods: Human cervical 'motion segments' (two vertebrae and intervening soft tissues) were obtained from cadavers aged 51-88yrs. Specimens were secured in cups of dental stone and subjected to static compressive loading (150N) for 20s. During this time, the distribution of vertically-acting compressive 'stress' was recorded along the postero-anterior diameter of the disc by pulling a 0.9mm-diameter pressure transducer through it (2). Injury was induced by compressing each specimen at 1mm/s while positioned in 20 deg of flexion, 15 deg of extension, or 8 deg of lateral bending. The distribution of compressive stress within the disc was then re-

measured. Specimens were sectioned at 2mm intervals in order to ascertain soft tissue disruption.

Results: In all six specimens tested to date, one or both of the apophyseal joint capsules were ruptured by the complex loading. Intervertebral disc prolapse also occurred in all six specimens, with the herniated nucleus appearing on the anterior, posterior and postero-lateral disc surface in extension, flexion and lateral bending respectively. All modes of failure affected intradiscal stresses: on average, nucleus pressure decreased by 75% (STD 7%), while stress concentrations in the annulus increased by 130% (STD 21%).

Discussion: These preliminary results confirm that severe complex loading can cause cervical discs to prolapse. No particular state of disc degeneration is required, provided the loading is sufficiently severe. Indeed, the altered stress distributions suggest that cell-mediated changes would probably follow prolapse.

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OC18

EVIDENCE FOR INVOLVEMENT OF A FACTOR X IN THE PROPAGATION OF THE OSTEOARTHRITIC PHENOTYPE

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Osteoarthritis (OA) is characterized by progressive erosion of articular cartilage due to degradation of the cartilage matrix. The major enzymes involved are the matrix metalloproteases and aggrecanases, which are either derived from the synovium or synthesized by chondrocytes as OA progresses. This abnormal enzyme synthesis is part of a phenotypic change from normal to 'degradative' chondrocytes. If this change could be prevented, then disease progression might be slowed.

In early OA, degradative chondrocytes are only present in the superficial zone, but with increasing severity of OA, more chondrocytes become degradative cells so that, in high-grade OA, these cells are also located in the deep zone. We hypothesized the existence of a 'factor X', which diffuses from the superficial to the deep zone and induces cells to change phenotype and express the proteases. We further hypothesize that this factor is released by degradative chondrocytes. To test the hypothesis, we co-cultured explants of human superficial-zone OA cartilage (which contains degradative cells and thus factor X) with explants of deep-zone cartilage from fracture neck of femur patients (#NOF), which contains mostly normal chondrocytes that do not express the proteases. We investigated MMP expression by real time RT-PCR and protein synthesis by immunohistochemistry.

Before culture, MMP-2, -3, -9, or -13 were expressed in the superficial-zone OA cartilage, but not in deep-zone #NOF cartilage, as expected. After 4 weeks with separate culture of superficial zones and deep zones, no MMPs was expressed in deep zone chondrocytes, suggesting that culture per se did not induce expression of these enzymes. Neither did culture abolish expression in the superficial zone, as confirmed by RT-PCR and immunohistochemistry. However, when superficial-zone cartilage was co-cultured with deep-zone cartilage, MMP-3 expression were induced in deep- zone chondrocytes, suggesting that a diffusible factor X, derived from degradative chondrocytes, had induced normal articular chondrocytes to express MMP-3. These experiments provide evidence for the existence of a factor that, when diffusing through the cartilage matrix, has the potential to induce normal non-enzyme expressing cells to become degradative chondrocytes.

OC19

DEFICIENCY OF THE SPECIFIC IMMUNE SYSTEM ENHANCES FRACTURE REPAIR IN-VIVO

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An estimated 10% of patients have problems with fracture healing. Initial studies have revealed that it is likely that both the innate and specific immune systems play a role in fracture repair, but this has not been attributed to particular components, cells or their products. It is known that the functionality of the immune system is impaired with age and this may account for the higher rate of delayed union in elderly patients.

We used a validated mouse model of a reproducible closed tibial fracture. In order to prevent any foreign body inflammatory/immune response no artificial internal fracture fixation was used and instead external support was provided using a Plaster of Paris cast. The role of the specific immune system was studied using an immunodeficient Balb/c SCID (Severe Combined Immuno Deficient) mutant mouse. The SCID mice were matched for age, sex (all males) and weight to the control, wild type Balb/c mice. Mechanical (4 point bending) and radiographic (Radiographs scanned and calculations of callus area, index and density made with image analysis software) measures were used to assess fracture repair at 21 days.

Mechanical measurements revealed an enhancement of fracture healing in the SCID mouse strain compared to the control strain, with stress at yield and Young's modulus higher in SCID mice than controls. (Stress at yield: 4.2 +/- 0.23MPa in Controls, 7.1 =/- 0.6MPa in SCIDs, P<0.01; Young' Modulus: 22.1 +/- 2.99MPa in Controls, 60+/- 9.9MPa in SCIDs P<0.01). There were no significant differences seen in mechanical properties of unfractured bone between the two strains. Radiographic analysis revealed no significant differences in callus area or index (both measurements of callus size) but callus density was significantly higher (P<0.01) in the SCID subjects compared to controls (2.6 +/- 0.06E5 Greyscale in SCIDs vs. 2.2 +/- 0.09E5 in controls).

We conclude that an abnormality of the immune system due to either lack of the specific immune system (T and B cells) or an enhancement of the innate system results in increased mineralization, stiffness and strength of fracture healing, and that further investigation might result in novel therapies directed toward avoidance of non/delayed-union.

OC20

PRIMARY FROZEN SHOULDER THAWED

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The purpose of the study was to test the hypothesis that cellular mechanisms of fibroblasts derived from primary frozen shoulder(PFS) exhibit similar activity in terms of contraction, response to cytokine (transforming growth factor-beta1) and mechanical stimulation similar to that generated by fibroblasts derived from dupuytren's disease. PFS is a debilitating disease of unknown aetiology, characterised by fibrosis with contracture of the coracohumeral ligament, tissues of the rotator interval and glenohumeral ligaments, leading to restrictive shoulder movements. Frozen shoulder has been postulated to be Dupuytren's disease of the shoulder with an association inferred since 1936.

Materials and Methods:

Primary explant cultures of fibroblasts from seven patients with PFS and five control patients were obtained using standard tissue culture techniques. Fibroblasts were seeded in 3-D collagen constructs and contraction force generated over 24hours measured using a culture force monitor(CFM) in real time. Increasing concentrations of TGF-beta1 were added to cell seeded gels and force generated measured using the CFM over 24hours. These mechanical output data were statistically compared to data available from Dupuytren's disease.

Results and Discussion:

Compared to Dupuytren's fibroblasts, PFS fibroblasts showed a statistically reduced ability to contract a 3-D collagen gel over 24hours (p<0.01). In Dupuytren's disease, fibroblasts derived from nodules and cords generate peak forces of 140dynes and 110dynes respectively, while PFS fibroblasts generated peak force of 8dynes The response to TGF-beta1 stimulation, which has been shown to enhance peak force contraction in Dupuytren's fibroblasts had no effect on PFS fibroblasts and this was statistically significant (p<0.01).

Conclusion:

These data suggest intrinsic differences in cellular activity and mechanisms between Dupuytren's and Primary Frozen Shoulder even though clinically they both manifest with a contracted extracellular matrix affecting function and requiring surgical intervention. This may explain increasing post surgically recurrence in Dupuytren's as compared to Primary Frozen Shoulder release.

OC21

HOW DO BMD, FRACTURE SEVERITY AND CEMENT TYPE INFLUENCE THE BIOMECHANICAL EFFECTS OF VERTEBROPLASTY?

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Introduction: We have shown that vertebroplasty increases stiffness and partly restores normal load-sharing in the human spine following vertebral fracture. The present study investigated how this restorative action is influenced by type of cement injected, bone mineral density (BMD), and fracture severity.

Methods: Fifteen pairs of thoracolumbar motion-segments (51-91 yrs) were loaded on a hydraulic materials testing machine to induce vertebral fracture. One from each pair underwent vertebroplasty with polymethylmethacrylate (PMMA) cement, the other with a biologically- active resin (Cortoss). Specimens were then creep loaded at 1.0kN for 2 hours. At each stage of the experiment, bending and compressive stiffness were measured, and 'stress' profiles were obtained by pulling a pressure-sensitive needle through the disc whilst under 1.5kN load. Profiles indicated the intradiscal pressure (IDP) and neural arch compressive load (FN). BMD was measured using dual photon X-ray absorptiometry. Severity of fracture was quantified from height loss. Changes were compared using repeated measures ANOVA.

Results: Fracture reduced bending and compressive stiffness by 31% and 41% respectively ($p<0.0001$), and IDP by 43%-62%, depending upon posture ($p<0.001$). In contrast, FN increased from 14% to 37% of the applied load in flexion, and from 39% to 61% in extension ($p<0.001$). Following vertebroplasty, these effects were significantly reversed, and in most cases persisted after creep-loading. No differences were observed between PMMA- and Cortoss-injected specimens. The decrease in IDP and increase in FN after fracture were correlated with BMD in flexion and with height loss in extension ($p<0.01$). After vertebroplasty, restoration of IDP and FN in flexion were correlated with their loss after fracture ($p<0.01$). The former was also related to BMD ($p<0.05$).

Conclusions: Changes in spinal load-sharing following fracture were partially restored by vertebroplasty, and this effect was independent of cement type. The effects of fracture and vertebroplasty on spinal load-sharing were influenced by severity of fracture, and by BMD. These findings suggest that people with more severe fractures and low BMD may gain most mechanical benefit from vertebroplasty.

OC22

OSTEOCYTES REPAIR FOLLOWING MICRODAMAGE INJURY IN VITRO

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Microcracks are known to accumulate in bone and are targeted for osteoclastic removal by a currently unknown mechanism. One potential targeting mechanism involves the production of signals from apoptotic osteocytes at the site of microdamage. The cause of osteocyte death close to microcracks is unknown but it has been suggested that it might involve the physical damage of the osteocyte itself. In light of a number of reports describing the ability of many cell types to repair and survive membrane rupture, here we have tested the ability of osteocytes to survive damage and estimated the rapidity of repair. We used a cell-based wounding model using a double labeling technique i.e. propidium iodide (PI) and fluorescent Dextran (FDx) to characterize osteocyte cell injury and repair, respectively. MLO-Y4 osteocytes were incubated with FDx and injury was induced using a scalpel blade. After 5 minutes the medium was replaced with fresh PI-containing medium and incubated for a further 5 minutes. FDx positive cells represented cells that had resealed and therefore retained FDx following plasma membrane rupture, whereas cells that were PI-positive had failed to reseal the membrane defect. Cell membrane repair was expressed as the percentage of resealed cells over the total number of injured cells (PI plus FDx positive cells). A cut line (less than 1micrometre in width) simulated a microcrack and induced various degrees of injuries to the cells by disrupting either the cytoplasmic processes or the cell body. Using the labelling technique we confirmed that the cut had disrupted the cell membrane and rendered it leaky. It was shown that a number of osteocytes were able to survive after either their body or cytoplasmic processes were severed 24 hours following the injury. Our result suggested that 42 % of the cells had repaired within 5 minutes of damage. These findings indicate that mechanically-wounded osteocytes are able to survive injury through plasma membrane resealing, as has previously been observed for other cell types. This finding may provide a new understanding of how osteocytes respond to potential injury following bone microdamage.

OC23

INSIGHT INTO THE FUNCTION OF BCL-2-ASSOCIATED ATHANOGENE-1 ANTI-APOPTOTIC PROTEIN DURING CHONDROCYTE DEVELOPMENT AND DIFFERENTIATION

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BAG-1 is a multifunctional pro-survival protein which enhances the anti-apoptotic activity of Bcl-2. BAG-1 knock-out mice die between E12.5- E13.5 due to apoptosis of haematopoietic and neural stem cells. In comparison to the widely studied role of Bcl-2 in maintaining the chondrocytic phenotype, function of BAG-1 in chondrocyte development remains poorly characterised. As a first step in elucidating BAG-1 function, we immunolocalised the protein, in long bone sections of 3-30 week BDF-1 mice, to the periosteum, osteoblasts of primary spongiosa, osteocytes in bone shaft, bone marrow, growth plate and articular chondrocytes. Murine BAG-1L (50 kDa) and BAG-1S (32 kDa) isoforms were detected, by Western blotting, in day 12 bone marrow cultures. BAG-1 expression altered dramatically with age in mouse growth plate and articular chondrocytes. In growth plates of young mice (3-5 weeks), BAG-1 expression was observed in reserve, proliferative and pre-hypertrophic chondrocytes. BAG-1 was detected only in pre-hypertrophic and hypertrophic chondrocytes in adult mice (10-15 weeks), while expression was restricted to upper hypertrophic zone chondrocytes in aged mice (20-30 weeks). Between 3-10 weeks, BAG-1 was expressed by most chondrocytes in the superficial and deep zones of articular cartilage. However, between 15-30 weeks, expression was restricted to chondrocytes of the superficial zone. A 28-day time-course of murine chondrocytic ATDC5 cells, differentiated in presence of insulin, was utilised as an in vitro model of endochondral ossification to monitor expression of BAG-1 in relation to chondrogenic differentiation markers, Sox-9, Bcl-2, Type II and X collagens. Expression of the BAG-1 gene, analysed by qPCR, and its protein isoforms (BAG-1L, BAG-1S), analysed by Western blotting, was maintained through the entire course of endochondral ossification, encompassing the various stages of chondrocyte differentiation until mineralization of cartilaginous nodules. The human BAG-1 promoter has four Sp1 transcription factor binding sites and one TGF (beta)-response element. Activity of the human pBAG-1-Luciferase reporter construct, transfected into ATDC5 cells, was down-regulated by 10 ng/ml recombinant human TGF-beta3 (a concentration used for chondrogenic induction and maintenance). Thus, BAG-1 is exquisitely expressed at different stages of chondrocyte differentiation during mouse development, and may represent a unique TGF (beta)-responsive, anti-apoptotic defence in chondrocyte differentiation.

OC24

ACTIVATION OF HUMAN OSTEOCLASTS BY CAPSAICIN

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Evidence available to date indicates that mild extracellular acidification is required for activation of resorption pit formation by osteoclasts (OC). However, it is still unclear whether pH sensor(s) expressed on or in OC are involved in mediating this activation response. One possible candidate for this role is the TRPV1 receptor. This multifunctional receptor responds not only to low pH but to capsaicin (the 'active ingredient' of chilli peppers) and heat, and has been implicated in bone cancer pain. We investigated the effects of capsaicin and capsazepine (a TRPV1 antagonist) on human OC formation and activation in control or acidified conditions. For the OC activation studies, human peripheral blood mononuclear cells (hPBMC) were cultured on ivory discs for 12d in MEM with FCS, M-CSF and RANKL at pH 7.4 and then exposed to 2nM-20 micromolar capsaicin at pH 7.4 or 6.9 for a further 2d. For the OC formation studies, hPBMC were cultured for 14d at pH 7.4 or 6.9 with 2nM-20

micromolar capsaicin. Mature OC were unaffected by capsaicin when pH was reduced to 6.9. However, at pH 7.4, OC resorptive activity was increased 4-fold by 2nM capsaicin, with maximal (5-fold) activation occurring at 200nM. The stimulatory action of capsaicin was equivalent in magnitude to that of low pH. In contrast, OC formation was inhibited 30-40% by capsaicin over the concentration range 2-200nM; continuous culture at pH 6.9 caused a similar reduction. Capsaicin was toxic to OC at concentrations >20micromolar. The stimulatory effect of low pH on OC resorption was halved by treatment with 10 micromolar capsazepine. RT-PCR analysis showed that the TRPV1 receptor was expressed by human OC, and was upregulated strongly by acidosis. Our results demonstrate that capsaicin, at hormonal concentrations, is a powerful, direct activator of normal human OC in non-acidified conditions, where 'classical' pro-resorptive factors such as PTH and RANKL are inactive. Furthermore, this activation response occurs at concentrations of capsaicin that are at least 2 orders of magnitude lower than those commonly used pharmacologically. Our findings suggest that the TRPV1 receptor could play a key role in OC activation and could offer a potential target for anti-resorptive drugs.

OC25

AN OSTEOPROTEGERIN-LIKE PEPTIDOMIMETIC (OP3-4) INHIBITS OSTEOCLASTIC BONE RESORPTION AND OSTEOLYTIC BONE DISEASE IN MULTIPLE MYELOMA

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Multiple myeloma is a B-cell malignancy characterised by the uncontrolled growth of plasma cells in the bone marrow, which results in increased osteoclastic bone resorption and bone destruction. Myeloma cells express the ligand for receptor activator of NFkappa-B (RANKL), induce RANKL expression in the bone marrow and down-regulate expression of the decoy receptor, osteoprotegerin (OPG), thereby promoting bone resorption. Targeting this system in myeloma has clear therapeutic potential. However, OPG also binds tumour necrosis factor-related apoptosis inducing ligand (TRAIL) and acts as a survival factor for myeloma cells by preventing TRAIL-induced apoptosis. Whether OPG can bind TRAIL and prevent apoptosis in vivo and the relative importance of OPG binding TRAIL and RANKL is unclear. In the present study we have investigated the ability of an OPG-like peptidomimetic (OP3-4), designed to block the RANKL/RANK interaction, to inhibit osteoclastic bone resorption and TRAIL-induced apoptosis in vitro and myeloma bone disease in vivo.

OP3-4 inhibited osteoclast formation (% TRAP positive cells, 0.0±0.1% vs 67.7±17.0%, $p<0.01$) and bone resorption (% dentine resorbed, 3.1±5.6% vs 90.0±36.9%, $p<0.01$) in a dose-dependent manner in vitro, as compared to vehicle control. Unlike OPG, OP3-4 had no effect on TRAIL-induced apoptosis of RPMI-8226 myeloma cells in vitro, as compared to vehicle control (94.7±4.0% vs 92.2±3.4%). Injection of 5T2MM murine myeloma cells into C57BL/KaLwRij mice resulted in the appearance of a serum paraprotein and the development of a bone disease characterised by increased osteoclastic resorption, a decrease in cancellous bone and the development of bone lesions on x-ray. Treatment of 5T2MM bearing mice with OP3-4, from the time of paraprotein detection, decreased the surface covered by osteoclasts (17.0±8.4% vs 24.9±8.7%, $p<0.05$), increased cancellous bone area (1.4±1.9% vs 0.5±0.6%) and prevented the development of lytic lesions (3.7±2.4 vs 7.5±4.1, $p<0.05$), when compared to vehicle control. OP3-4 also reduced tumour burden (59.0±31.9% vs 86.1±23.0%, $p<0.05$) compared to vehicle control.

These data suggest that OP3-4, and the selective inhibition of RANKL but not TRAIL activity, is effective in preventing myeloma bone disease and offers a novel therapeutic approach to treating this aspect of myeloma.

OC26

IN VIVO STIMULATION OF BONE RESORPTION BY APOPTOTIC OSTEOCYTES

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Very little is known about the signals involved in the targeted bone remodelling process. Recently it was shown that osteoclastic removal of microdamage was preceded by osteocyte apoptosis in the region of damage indicating that signals released by apoptotic osteocytes could drive bone resorption in health and disease. Here, we studied the effects of apoptotic osteocyte-derived signals on osteoclast formation and bone resoprtion by injecting osteocyte apoptotic bodies (OAB) onto the forming or quiescent surface of murine calvariae in vivo.

Injection occurred on the right side parietal bone, at approximately 3 mm away from the edge of the bone, while the left (noninjected) side was used to provide nontreated controls. In order to compare the effects of OAB on osteoclast formation to that of apoptotic bodies of alternative bone cell origin, calvariae were also injected with primary murine osteoblast apoptotic bodies (AB). Calvariae were injected once and animals were sacrificed 5 days following treatment.

Calvarial surfaces injected with either vehicle or osteoblast AB as well as the non-injected (left) side of the parietal bone, appeared smooth and covered by lining cells with no evidence of TRAP positive osteoclasts. However, in response to exposure to OAB for 5 days, the previously forming surface was characterised by the presence of TRAP positive osteoclastic cells and associated resorption lacunae. Quantification of the percentage resorption surface demonstrated a large, 340-fold, increase in resorption of bone close to the injection site, compared to vehicle (approximately 3 mm away from the edge of the right parietal bone).

Here we have targeted in vivo the activity of osteoclasts and/or their precursors onto a specific bone surface through the application of OAB to that particular site. Hence, the region specific apoptotic death of osteocytes in our bones might underlie the mechanism by which targeted remodelling occurs in bone.

OC27

A NOVEL ROLE FOR IL-23 IN OSTEOCLAST DEVELOPMENT

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IL-23 is a novel cytokine belonging to IL-12 family that plays an important role in Th-1 cell development and in inflammation mediated disease processes associated with bone loss including rheumatoid arthritis and periodontitis. IL-23 is a heterodimeric protein consisting of the IL-12 p40 subunit and a novel polypeptide chain termed IL-23 p19. We have shown that stimulation of osteoblasts derived from bone marrow stromal cells with bacterial endotoxin (LPS) induces the expression of both IL-23 subunits, IL-12 p40 and IL-23 p19. To investigate the role of IL-23 in inflammatory bone loss, we analysed the effect of IL-23 on RANKL-mediated osteoclastogenesis from mononuclear precursor cells isolated from bone marrow and spleen of DBA-1 mice. Recombinant IL-23 was produced in CHO cells from a cDNA encoding the IL-12 p40 subunit connected to the IL-23 p19 subunit via an oligo(GGGGS) linker and shown to induce IFN-a production in CD4+ T-cells. Formation of functional osteoclasts was analysed by identifying multinucleated cells displaying TRAP activity and using the dentine slice pit formation assay. IL-23 dose-dependently promoted osteoclastic differentiation at suboptimal concentrations of RANKL whereas IL-12 had no effect. IL-23 alone was not sufficient to induce osteoclast formation. Analysis of osteoclast formation from the RAW 264.7 macorphage precursor line demonstrated that IL-23 acts directly on osteoclast precursors. IL-23 upregulated RANK expression in early osteoclast precursors. Therefore synergistic action of IL-23 and RANKL may relate to increased NF-kB activation by direct signalling or indirectly by increasing RANK expression. Injection of IL-23 into DBA-1 mice accelerated joint destruction in the collagen-induced arthritis model. The results from this study show a novel role for IL-23 in osteoclast development. Increased production of IL-23 associated with inflammation may therefore contribute to bone loss by increasing development of osteoclasts from precursors.

OC28

IMAGING OF THE MUSCULOSKELETAL SYSTEM USING 3D ULTRASOUND

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Imaging of the musculoskeletal system is vital for delivering optimum treatment particularly in the assessment of fracture healing. X-ray and CT are adequate imaging methods for bone but, soft tissue needs other modalities such as MRI and Ultrasound. We propose the use of Freehand 3D Ultrasound to study the early stages of fracture healing by imaging the bone surfaces around the fracture site and monitoring changes in the surrounding soft tissue.

Freehand 3D ultrasound is acquired by attaching a position sensor to the probe of a conventional 2D diagnostic ultrasound machine. As the probe is moved, its position and orientation are recorded along with the 2D ultrasound images. This enables slices through the body to be viewed that would be inaccessible using a normal ultrasound system. Bone surfaces around a fracture site are scanned and the data reconstructed using the Stradx and Stradwin software developed by Cambridge University, to give a 3D visualization of the area.

To assess the feasibility of this proposed method the lower limbs of healthy volunteers were scanned using a 5-10MHz ultrasound probe. The scanning resolution of the system was evaluated using a phantom to ensure millimetre detail could be detected as would be required for imaging early fracture healing. It was found that detail down to 0.8mm could easily be resolved for measurement.

The 3D system could accurately profile the different soft tissue interfaces. The visible surfaces of the tibia were reconstructed to give 3D models. Additional layers of soft tissue interfaces could easily be added to these models to provide more detail.

This imaging modality can provided detailed 3D models of bone the bone surface and surrounding soft tissue. As ultrasound is non-ionizing, rescanning can be conducted more frequently than with CT or x-ray thus offering a more accurate assessment of a patient's response to healing.

OC29

A NEW UNDERSTANDING OF STRUCTURAL ADAPTATION OF TENDON TO MECHANICAL LOAD

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The skeletal system exhibits functional adaptation. For bone the mechanotransduction mechanisms have been well elucidated; in contrast, the response of tendon to its mechanical environment is much more poorly understood despite tendon disorders being commonly encountered in clinical practice. This study presents a novel approach to developing an isolated tendon system in vivo. This model is used to test the hypothesis that stress-shielding, and subsequent restressing, causes significant biomechanical changes. We propose a control mechanism that governs this process.

A custom-built external fixator was used to functionally isolate the ovine patellar tendon(PT). In group 1 animals(n=5) the right PT was stress-shielded for 6 weeks. This was achieved by drawing the patella towards the tibial tubercle, thus slackening the PT. In group 2 (n=5) the PT was stress-shielded for 6 weeks. The external fixator was then removed and the PT physiologically loaded for a further 6 weeks. In each case, the PT subsequently underwent tensile testing and measurement of length(L) and cross-sectional area(CSA). The untreated left PTs acted as controls (n=10).

6 weeks of stress-shielding significantly decreased material and structural properties of tendon compared to controls (elastic modulus(E) 76.2%, ultimate tensile strength(UTS) 69.3%, stiffness(S) 79.2%, ultimate load(UL) 68.5%, strain energy(SE) 60.7%; $p<0.05$). Ultimate strain(US), L and CSA were not significantly changed. 6 weeks of subsequent functional loading (Group 2) caused some improvement in material properties, but greater recovery in structural properties (E 79.8%, UTS 91.8%, S 96.7%, UL 92.7%, SE 96.5%). CSA was significantly greater than Group 1 tendons at 114% of control value.

Previous models of tendon remodelling have relied on either joint immobilization or direct surgical procedures. This model allows close control of the tendon's mechanical environment whilst allowing normal joint movement and avoiding surgical insult to the tendon itself. The hypothesis that stress-shielding, and subsequent restressing, causes significant biomechanical changes has been upheld. We propose that the biomechanical changes observed are governed by a strain homeostasis feedback mechanism.

OC30

A NEW METHOD TO MEASURE THE INTER-FRACTURE SITE MOVEMENTS (IFMS) DYNAMICALLY BY MEANS OF STEWART PLATFORM ALGORITHM

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The Ilizarov technique of distraction osteogenesis is becoming a more common way of treating complicated fractures. It has been shown that shear IFMs will delay bone healing whilst axial IFMs are beneficial to the bone healing. Therefore to measure IFMs in conditions of mobility will provide critical information for research and clinic diagnosis. Such data are not provided by static measurements. Traditionally the IFMs were measured by implanting transducers to the bone or using radiological methods. However, these methods are not suitable for either clinic utilization or measurement of IFMS when patients are doing movements which simulate their daily activities. We have designed a dynamic IFMs measuring device.

It includes a displacement transducer array, which is connected to the Ilizarov wires. This transducer array consists of 6 parallel linear displacement transducers, each of which is attached to the fixing wires of the fixator. This arrangement of transducers can fit into the configuration of Stewart Platform. The Reverse Stewart Platform algorithm was employed to calculate IFMs. Without measuring the bone fracture segments directly, the two segments were fitted into two planes virtually. By studying the relative movements of the two virtual planes, the algorithm transfers the relative movement to relative axial &shear translation, and relative bending & torsion rotation, between the two fracture segments. Wireless interface was used to transfer the displacement readings from the transducer array to the computer. This setup allows patient perform activities which represent their routine activities.

In laboratory studies, we found the error of this system to be related to the IFMs. For small movements around 100 micron, the absolute error was 50 micron, whereas for larger movements around 1 mm, the error was within 0.22mm.

This real time monitoring method will allow kinematical and kinetic studies on fracture patients treated with Ilizarov frame. Measurements obtained using this novel device will reflect the natural pattern of IFMs during the patients' daily life. Since use of the device requires no additional pin, wire or operative procedure, it will be clinically applicable. The accurate real-time IFMs measurements will help elucidate the complex interplay between movement and bone formation.

OC31

EXPRESSION PROFILING OF MATRIX METALLOPROTEINASES IN DUPUYTREN'S DISEASE

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The matrix metalloproteinases (MMPs) and ADAMTSs (a disintegrin and metalloproteinase with thrombospontin motif) are related enzymes collectively responsible for turnover of the extracellular matrix. The balance between the proteolytic action of the MMPs and ADAMTSs, and their inhibition by the tissue inhibitors of metalloproteinases (TIMPs), underpins many pathological processes. Deviation in favour of proteolysis is seen in e.g. invasive carcinoma, whereas an imbalance towards inhibition causes e.g. fibrosis.

Dupuytren's Disease (DD) is a fibroproliferative disorder affecting the palmar fascia, leading to contractures. A group of patients with end-stage gastric carcinoma were treated with a broad spectrum MMP inhibitor in an attempt to reduce the rate of tumour advancement: a proportion developed a 'musculoskeletal syndrome' resembling DD. Several groups have looked at subsets of the metalloproteinase family in relation to DD, but to date, a study of the gene expression of all of the members has not been published. We therefore set out to profile

the mRNA expression for the 23 known MMPs, 4 TIMPs & 19 ADAMTSs in DD and normal palmar fascia.

Tissue samples were obtained from patients undergoing surgery to correct contractures caused by DD and from healthy controls undergoing carpal tunnel decompression. The DD tissue was separated macroscopically into cord and nodule. Total RNA was extracted and mRNA expression analysed by quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR), normalised to 18S rRNA. Comparing across all genes, the DD nodule, DD cord and normal palmar fascia samples each had a distinct mRNA expression profile. Statistically significant ($p<0.05$) differences in mRNA expression included: higher MMP-2, -7 and ADAMTS-3 levels in both cord and nodule; higher MMP-1, -14, TIMP-1 and ADAMTS-4 and -5 in nodule alone, lower MMP-3 in nodule and cord and lower TIMP-2, -3 and -4 and ADAMTS-1 and -8 levels in nodule alone.

The distinct mRNA profile of each group suggests differences in extracellular proteolytic activity which may underlie the process of fascial remodelling in DD. Further in vitro experiments are planned based on these observed differences in gene expression.

OC32

THE MOLECULAR AND CELLULAR RESPONSE OF HUMAN BONE TO MECHANICAL STIMULATION

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Physical activity is a key determinant of bone mass and health, however during adulthood and ageing there appears to be a decrease in the ability to respond positively to exercise which is variable between individuals. While exercise is known to protect against the osteoporotic process with modest increases in BMD the exact cellular and molecular responses are poorly understood. We have studied the effect of mechanical stimulation on bone histomorphometric parameters, osteocyte viability and gene expression in human trabecular bone maintained in a 3D bioreactor.

Trabecular bone cores were prepared from femoral head tissue removed from patients undergoing total hip arthroplasty and maintained in the bioreactor system for 3 (n= 4 patients) , 7 (n=5 patients) or 28 days (n=1 patient). Cores (n=3 per patient) were either frozen directly on preparation (T0), placed in the bioreactor system and subjected to Mechanical stimulation (3000 µstrain in jumping exercise waveform repeated at 1Hz for 5 minutes daily) or maintained in the bioreactor system with no mechanical stimulation as control. After the experimental period total cell numbers, cell viability and apoptosis were determined in un-decalcified cryosections at specific distances throughout the bone cores by nuclear staining (DAPI), lactate dehydrogenase activity (LDH) and Nick Translation Assay respectively. Consecutive sections were collected and RNA extracted for gene expression analysis.

Mechanical stimulation was shown to increase Bone Formation Rate (BFR) as determined by Calcein label/distance to bone surface in the 28 day experiment (BFR mcm/day Control 0.01 ± 0.0035 vs Load 0.055 ± 0.0036 p=0.0022). Expression of bone formation markers such as Alkaline Phosphatase and Collagen Type I was shown to increase in all patients however there was an individual variation in the response of Osteopontin to mechanical stimulation as determined by quantitative real time PCR expression analysis. Numbers of viable osteocytes at T0 varied between individual patients however viability was significantly increased and apoptosis decreased in association with mechanical stimulation compared to control in all patient samples examined ($p \leq$ to 0.021). Our data tend to support animal model findings relating to the osteocyte saving effects of exercise and provide an insight into the molecular detail of the exercise response in human bone.

OC33

SINGLE INJECTION OF THROMBIN-RELATED PEPTIDE (TP508) IN A SLOW-RELEASING PREPARATION ENHANCED BONE CONSOLIDATION DURING DISTRACTION OSTEOGENESIS

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The thrombin-related peptide, TP508, a synthetic 23 amino acid peptide, has been shown to promote soft tissue, cartilage and fracture repair. We have previously demonstrated that two injections of TP508 have significantly enhanced bone consolidation in a rabbit model of distraction osteogenesis. This study was to test if a single injection of TP508 in a slow-releasing preparation will have the similar effects.

Unilateral tibial osteoectomies were stabilized with M100 Orthofix lengtheners in 17 male adult NZW rabbits. After 7 days, lengthening was initiated at a rate of 1.4 mm/day for 6 days. The following treatments were given: Group 1: TP508 in saline (300ug/300ul, n=6) was injected into the osteotomy gap at day of surgery and into the lengthening gap at end of lengthening. Group 2 (Control): Dextran gel (300ul, n=6) and Group 3: 300ul Dextran gel mixed with microspheres containing 300ug TP508 (n=5), was injected into the lengthening gap at end of lengthening. All animals were terminated 2 weeks after lengthening. Bone formation was assessed by weekly radiography and the specimens were subject to pQCT, microCT and histology examinations.

On radiographies there was more bone formation in the TP508 treated groups than that of the control group at 1st week post-lengthening and complete union was seen in 50% rabbits in Group 1, 33% in Group 2, and 60% in Group 3 at termination. The mean BMD of the regenerates was significantly higher in the TP508 treated groups than that of the control group ($p<0.05$). MicroCT analysis demonstrated advanced bone formation in the TP508 treated animals. For histology, the regenerates were mainly consisted of woven bone of neocortilization and callus remodelling in Groups 1 and 3, whereas in Group 2, focal defects with cartilaginous tissues were frequently seen.

In conclusion we have demonstrated that a single injection of TP508 in the form of slow releasing microspheres has enhanced bone consolidation during distraction osteogenesis. TP508 may therefore be applied in the slow-releasing preparation for augmenting bone formation at reduced doses, costs and risks of infections through repeated injections.

OC34

BIOMATERIAL SURFACE ARCHITECTURE DICTATES CELL:CELL INTERACTIONS AND REGULATES OSTEOBLAST DIFFERENTIATION

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One way to improve orthopaedic materials is to understand the exact architectural parameters that influence bone cell behaviour. In this study substrates with highly controlled surface features were created using photolithographic processes. These surfaces were contrasted for their ability to influence osteoblast activity and intercellular communication.

An etched silicon wafer was created by photolithography and used to hot-emboss grooved substrates (10-30micrometers wide/ 5-16micrometers deep) in poly-carbonate (PC). Smaller features were created on polydimethylsiloxane (PDMS) by casting over a photoresist patterned silicon wafer. Rat osteoblasts were routinely cultured on flat or micro-fabricated substrates or in media supplemented with osteogenic stimuli for 35 days. Alkaline phosphatase activity was colourimetrically localised, and mineralised matrix visualised with Von Kossa staining. Connexin-43 was immunolocalised with a CY-2 conjugated antibody. Intracellular communication was studied using a dye (Lucifer yellow) transfer technique and fluorescence microscopy.

Osteoblasts were aligned on the grooved surface. In 10micrometers grooves, cells were in single rows while at 30micrometers the rows were two/three cells wide. Culture of osteoblasts on these surfaces under osteogenic conditions demonstrated alkaline phosphatase activity comparable to flat surfaces but after 28-35 days there was little evidence of bone-like nodules on the grooved substrates. We

hypothesized that on grooved substrates cell:cell communication is compromised thus gap-junctions were studied. Image analysis showed that there was lower connexin-43 expression in cells on grooved substrates and fewer discrete gap junction complexes compared to flat surfaces ($p<0.05$ ANOVA.). There were also differences between the grooves with connexin-43 most abundant on the widest (30micrometers) and deepest grooves (16micrometers). Analysis of dye transfer demonstrated that whilst cell:cell coupling was maintained within grooves it was reduced at the boundaries of the groove. A surface of asymmetric arrays of micro-columns (diameter 5micrometers) was fabricated to retain lateral interactions between osteoblasts whilst still aligning cells. Osteoblast differentiation now resulted in the formation of numerous bone-like nodules and matrix was aligned in the direction of the shortest column distances.

Maintaining appropriate cell:cell communication structures is pivotal in the process of osteoblast differentiation and the design of novel biomaterial surfaces should ensure that cells can maintain these critical interactions.

OC35

AN INNOVATIVE EX VIVO MODEL FOR CHONDROGENESIS AND OSTEOGENESIS

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Cartilage and bone degeneration are major healthcare problems affecting millions of individuals worldwide. Elucidation of the processes modulating the cell-matrix interactions involved in cartilage or bone formation offer tremendous potential in the development of clinically relevant strategies for cartilage and bone regeneration. We have therefore adopted an ex vivo tissue engineering approach to investigate chondrogenesis and osteogenesis using a mix human mesenchymal progenitor populations encapsulated in biomineralised polysaccharide templates with or without the addition of type-I collagen.

Alginate/chitosan polysaccharide capsules containing 2.5mg/ml type-I collagen and TGF-beta-3 were encapsulated with human bone marrow cells (HBMC), articular chondrocytes or a co-culture at a ratio of 2:1 respectively and placed in a rotating (Synthecon) bioreactor or held in static 2D culture conditions for 28 days, to determine whether the presence of type-I collagen within the alginate could promote the synthesis of an extracellular matrix.

Constructs were stained with alcian blue, sirius red and von Kossa. In bioreactor samples encapsulated with HBMC and type-I collagen, viable cells were present within lacunae, surrounded by a matrix of proteoglycans and fibrous collagen, which was mineralized. Immunohistochemistry and polarised light microscopy indicated an organised collagenous matrix with extensive expression of type I collagen and bone sialoprotein with small regions of type II collagen. Type X collagen was also expressed indicating the presence of hypertrophic chondrocytes. Within the static HBMC groups, smaller areas of matrix were generated with decreased expression of type-I and type-II collagen. Co-culture bioreactor samples also demonstrated regions of new mineralised bone matrix; however these were less prominent than in the HBMC only groups. No matrix formation was observed in chondrocyte cultures although the cells remained viable as assessed by live/dead staining. Biochemical analysis indicated significantly increased ($p<0.05$) DNA in all bioreactor samples in comparison with static constructs and significantly increased protein in HBMC bioreactor constructs in comparison with other cell types.

These studies outline a unique tissue engineering approach, utilizing individual and mixed human mesenchymal progenitor populations coupled with innovative polysaccharide templates containing type I collagen and bioreactor systems to promote chondrogenic and osteogenic differentiation.

OC36

AUGMENTATION OF ALLOGRAFT WITH HUMAN BONE MARROW STROMAL CELLS: VALIDATION OF CELL SURVIVAL, PROLIFERATION, OSTEOGENIC PHENOTYPE AND MECHANICAL STRENGTH

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The use of fresh morsellised allograft in impaction bone grafting for revision hip surgery remains the gold standard. Bone marrow contains osteogenic progenitor cells that arise from multipotent mesenchymal stem cells and we propose that in combination with allograft will produce a living composite with biological and mechanical potential. This study aimed to determine if human bone marrow stromal cells (HBMSC) seeded onto highly washed morsellised allograft could survive the impaction process, differentiate and proliferate along the osteogenic lineage and confer biomechanical advantage in comparison to impacted allograft alone. Future work into the development of a bioreactor is planned for the potential safe translation of such a technique into clinical practice.

Methods: HBMSC were isolated and culture expanded in vitro under osteogenic conditions. Cells were seeded onto prepared morsellised allograft and impacted with a force equivalent to a standard femoral impaction (474J/m2). Samples were incubated for either two or four week periods under osteogenic conditions and analysed for cell viability, histology, immunocytochemistry, and biochemical analysis of cell number and osteogenic enzyme activity. Mechanical shear testing, using a Cam shear tester was performed, under three physiological compressive stresses (50N, 150N, 250N) from which the shear strength, internal friction angle and particle interlocking values were derived.

Results: HBMSC survival post impaction, as evidenced by cell tracker green staining, was seen throughout the samples. There was a significant increase in DNA content ($P<0.05$) and specific alkaline phosphatase activity ($P<0.05$) compared to impacted seeded allograft samples. Immunocytochemistry staining for type I collagen confirmed cell differentiation along the osteogenic lineage. There was no statistical difference in the shear strength, internal friction angle and particulate cohesion between the two groups at 2 and 4 weeks.

Conclusion: HBMSC seeded onto allograft resulted in the formation of a living composite capable of withstanding the forces equivalent to a standard femoral impaction and, under osteogenic conditions, differentiate and proliferate along the osteogenic lineage. In addition, there was no reduction in aggregate shear strength and longer term studies are warranted to examine the biomechanical advantage of a living composite. The therapeutic implications of such composites auger well for orthopaedic applications.

OC37

MRNA EXPRESSION OF HUMAN PLACENTAL CALCIUM TRANSPORTER (PMCA3) PREDICTS INTRAUTERINE BONE MINERAL ACCRUAL IN THE OFFSPRING

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Evidence suggests that intrauterine bone mineral accrual predicts osteoporosis risk in later life, and that maternal 25(OH)-vitamin D status in pregnancy is a determinant of neonatal bone mass. In this study, we aimed to explore the relationship between intrauterine bone mineral accrual in the offspring, and expression of calcium transporters in the human placenta.

Healthy, term pregnancies were recruited from the Southampton Women's Survey, a unique, ongoing, well-established cohort of women, aged 20-34 years, assessed before and during pregnancy. Tissue samples from 70 placentae were rapidly frozen in liquid nitrogen and stored at -70 degrees C. A quantitative real time polymerase chain reaction was used to measure the mRNA expression of PMCA isoforms 1, 3 and 4, using beta-actin as a control gene. Neonatal whole body bone area, mineral content and density (BA, BMC, BMD) were measured within 20 days of birth using a Lunar DPX DXA instrument. Linear regression methods were used to explore the relationship between PMCA expression and neonatal bone mass.

After controlling for beta-actin expression, PMCA3 mRNA expression predicted neonatal WB BA (r=0.28, p=0.02), WB BMC (r=0.25, p=0.04), placental weight (r=0.26, p=0.03), and birthweight (r=0.33, p=0.006). There were no associations between PMCA1 and 4 and neonatal outcomes; however, mRNA levels of these two transporters were positively predicted by paternal height (PMCA1: r=0.40, p=0.01, PMCA4: r=0.38, p=0.02). In a multivariate model, the relationship between placental PMCA3 expression and neonatal BMC was independent of maternal height, and pre-pregnant fat stores, parity, smoking, and calcium intake (p~0.05).

Expression of the placental calcium transporter PMCA3 predicts neonatal whole body bone mineral content. This relationship may explain, in part, the mechanism whereby a mother's 25(OH)-vitamin D stores influence her offspring's bone mass. The associations between PMCA1 and 4 and paternal, but not maternal, height suggest that these genes may be imprinted. Further elucidation of this process may allow development of novel therapeutic strategies to optimise childhood bone mineral accrual and thus reduce osteoporotic fractures in future generations.

OC38

GROWTH IN INFANCY AND CHILDHOOD PREDICTS HIP FRACTURE RISK IN LATE ADULTHOOD IN MEN AND WOMEN

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Although peak bone mass accounts for up to 60% of adult bone mass variance, it is not known whether differences in peak bone mass lead to differences in adult fracture risk. We have previously demonstrated that poor growth in late childhood was associated with an increase in adult hip fracture risk. Using a second cohort, we have investigated the relationship between growth in early childhood and later fracture risk.

Maternity, school and welfare records were electronically linked with hospitalization and mortality records in Helsinki, Finland. 13, 345 individuals (6,370 women) born in Helsinki between 1934-1944 were identified. These individuals had weight and length recorded at birth as well as during infancy and childhood. Hip fractures were identified using hospitalization and mortality records.

From 13,345 individuals, 106 hip fractures were identified. The weight and BMI at one year and the change in body build (BMI) from years 1 to 12 were strongly associated with adult hip fracture risk. Those in the lowest quarter of BMI gain had a 2.2 fold increase in hip fracture risk (p=0.05) compared with those in the highest quarter of this distribution. The increased risk was present only in women. In males, higher adiposity in the first year of life was associated with an increased risk of hip fracture (p=0.019). While there was no significant association with maternal height, increasing maternal BMI was independently associated with an increased risk of adult hip fracture in her offspring.

We have been able to demonstrate that change in childhood adiposity is associated with an increased risk in hip fracture and the mechanism appears to be mediated via different growth trajectories in early childhood.

OC39

A RATIONALE FOR TREATING LEG LENGTH DISCREPANCY USING PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) involves light activation of photosensitizing compounds, culminating in the generation of cytotoxic, reactive oxygen species. This study investigates the use of PDT in regulating bone development with a view to its potential role in treating Juvenile leg length discrepancy (LLD). Transgenic mice expressing the luciferase firefly gene upon activation of a promoter sequence specific to the vascular endothelial growth factor (VEGF) gene were subject to benzoporphyrin derivative monoacid (BPD-MA)-mediated PDT in the right, tibial epiphyseal growth plate at the age of 3 weeks. BPD-MA was administered intracardially (2mg/kg) followed 10 mins later by a laser light (690 +/- 5 nm) at a range of doses (5-27J, 50 mW output) delivered either as a single or repeat regimen (x2-3). Contra-lateral legs served as no-light controls. Further controls included animals that received light treatment in the absence of photosensitizer or no treatment. Mice were imaged for VEGF related bioluminescence (photons/sec/steradian) at t= 0, 24, 48, 72 h and 1-4 weeks post PDT. FaxitronÔ x-ray images provided accurate assessment of bone morphometry. Upon sacrifice, the tibia and femur of the treated and untreated limbs were harvested, imaged and measured again and prepared for histology. A number of animals were sacrificed at 24 h post PDT to allow immunohistochemical staining for CD31, VEGF and hypoxia-inducible factor (HIF-1 alpha) within the bone. PDT-treated (10 J, x2) mice displayed enhanced bioluminescence at the treatment site (and ear nick) for up to 4 weeks post treatment while control mice were bioluminescent at the ear-nick site only. Repeat regimens provided greater shortening of the limb than the corresponding single treatment. PDT-treated limbs were shorter by 3-4 mm on average as compared to the contra lateral and light only controls (10 J, x2). Immunohistochemistry confirmed the enhanced expression VEGF and CD31 at 4 weeks post-treatment although no increase in HIF-1 alpha was evident at either 24 h or 4 weeks post PDT treatment. Results confirm the utility of PDT to provide localized effects on bone development for minimally invasive treatment of LLD that may also be applicable to other related skeletal deformities.

OC40

REMODELLING CLUSTERS REDUCE LOCAL BONE STRAIN AND FAVOUR CANAL MERGING

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Cortical remodelling in the femoral neck, shaft and ilium is spatially clustered and not random. Excessive osteoclastic resorption within these clusters probably merges individual canals to form large composite canals decreasing bone strength. We have previously hypothesised that reductions in osteocytic NO generation is a mechanism for canal merging but doubts have been raised as to whether osteocytes produce sufficient NO to inhibit osteoclastic resorption. We have now investigated, using finite element analysis, whether clustered remodelling and the development of composite canals alters the local strain environment so as to favour canal merging and continued enlargement of the resultant composite canals.

Binary images of cortex containing just normal canals (7 images), remodelling clusters (n=4) or composite canals (n=5) were imported into Femlab, converted into a mesh of triangular elements with material properties of human bone. The distributions of the principal strains in 3D were modelled by constraining two adjacent edges of the 2D image in the plane of the section and applying a 0.1% compressive strain to each of the other edges. Differences in the areas of bone (as a proportion of bone area) within each of 7 equal bands of strain ranging from high compression (-0.04 to -0.008%) to neutral to tensile (0 to 0.0005%) were analysed using JMP software. To investigate the effect of increased cortical porosity the canals in the normal canal images were separately dilated (20 micrometers) and the effect on strain densities re-analysed.

Clustering of remodelling canals resulted in a significant shift towards a neutral strain environment. This was greatly increased in bone containing composite canals. (High Compression:- normal: 0.35±0.09, cluster: 0.08±0.08, composite: 0.002±0.001; Neutral Strain:- normal: 0.08±0.02, cluster: 0.24±0.07, composite 0.44±0.08). Increasing porosity by expanding individual canals induced a slight shift to lower strains that was not as marked as that for clustered canals.

Reducing local bone strain leads to bone resorption. This study indicates that the presence of a remodelling cluster reduces the local strain between the individual canals favouring bone resorption between them. Once a composite canal has been formed the further reductions in local strain favour enlargement of that canal.

OC41

ACTIVATION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR DELTA (PPARDELTA) INCREASES PERIOSTEAL BONE FORMATION

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PPARs are a family of nuclear receptors that upon activation by an appropriate ligand, form a heterodimer complex with retinoic acid receptor and regulate transcription of target genes. Previously we have shown that activation of PPARdelta by linoleic acid or bezafibrate increases CFU-Ob formation.

The effect of the PPARdelta agonists was examined in vivo. Briefly, intact male rats were injected daily s.c. with linoleic acid, or bezafibrate for 12 weeks. Linoleic acid (0.3 mg/kg/d) and bezafibrate (1 mg/kg/d) increased metaphyseal BMD by approximately 8%. Ex vivo osteoprogenitor number was increased following treatment as demonstrated using the CFU-f assay. Serum osteocalcin increased but there was no change in serum TRAP5b.

Compared with the vehicle control group, both treatment groups had significantly increased trabecular bone area, trabecular number at the proximal metaphysis. Linoleic acid treatment increased BFR by approximately 25% whereas bezafibrate treatment decreased BFR by approximately 44%. Neither treatment affected osteoclast number or surface.

Cross-sectional measurements at the tibial-fibula junction showed that treated animals had considerably larger bones than control animals. Total tissue area increased from 4.5 mm2 to 6.0 mm2 and 5.9 mm2. There was no difference in medullary cavity area or endocortical perimeter. Cortical bone area significantly increased from 3.66 mm2 in vehicle-treated animals to 5.05 mm2 and 5.03 mm2. Similarly periosteal perimeter increased significantly. In contrast to trabecular BFR, there was a 2-3 fold increase in periosteal-BFR in both treatment groups.

In conclusion, we have demonstrated that activation of the PPAR delta results in an increase in periosteal bone formation.

OC42

NEW SCAFFOLDS FOR SKELETAL REGENERATION: STIMULATING ANGIOGENESIS AND OSTEOGENESIS

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Bone is a highly complex, vascularised tissue, which is continually being remodelled. With an increasing ageing population, strategies to augment bone formation as a consequence of disease or trauma, for skeletal repair, remain an unmet clinical need. Regenerative strategies for the stimulation of osteogenesis have received considerable attention however; functional blood vessels are needed to provide oxygen and nutrients to maintain the growth of engineered scaffolds in vivo. Key in the role of angiogenesis is vascular endothelial growth factor (VEGF). Using supercritical fluid CO2 (scCO2) mixing technology, porous poly(DL-lactic acid) (PDLLA) scaffolds have been encapsulated with Bone Morphogenic Protein-2 (BMP-2) and VEGF. We investigated whether the release of encapsulated factors from scaffolds could stimulate both osteogenesis and angiogenesis respectively.

The activity of scaffolds encapsulated with human BMP-2 was determined by induced expression of Alkaline Phosphatase (AP) in the C2C12 promyoblast cell line. The activity of scaffolds encapsulated with VEGF was determined by the angiogenic tubule formation of human umbilical vein endothelial cells (HUVECs) on a growth factor reduced Matrigel matrix and by the chorioallantoic membrane assay (CAM).

BMP-2, which was subjected to scCO2, maintained its bioactivity to increase AP activity in vitro (nMol PNPP/µg DNA). Control = 2.5±1.3; ***BMP-2 (100ng/ml) = 8.9±3.6 ***ScCO2 treated BMP-2 = 7.7±3.5, (***P<0.001 Mean± S.D (n=3). The encapsulated BMP-2-PDLLA scaffolds showed significant release of the growth factor over a 28 day period in culture. VEGF subjected to scCO2, maintained its bioactivity to increase the length of endothelial microtubules following HUVECs cultured on Matrigel. Endothelial tube lengths (µm/field) Control=1237±179; *VEGF(10ng/ml)=1937±205; *ScCO2VEGF(10ng/ml)=2085±234, (*P<0.05; n=3 ± SEM). VEGF release from PDLLA scaffolds increased blood vessel formation in the chick chorioallantoic membrane assay. Number of vessels, PDLLA=24.8±9.7; **ScCO2-PDLLA-VEGF=44.1±12.1. (**P<0.01 Mean±SD)

We demonstrate that encapsulated BMP-2 and VEGF PDLLA scaffolds maintain there bioactivity and can stimulate osteogenesis and angiogenesis in vitro. These intelligent growth factor delivery scaffolds, which offer a unique approach to co-ordinate angiogenesis and bone formation offer innovative approaches to advance our ability to engineer vascularised bone tissue. This will prove pivotal for skeletal repair in patients suffering from bone loss as a consequence of trauma, ageing and disease.

OC43

ENHANCED CHONDROGENESIS OF INFRAPATELLAR FAT PAD STEM CELLS IN HYPOXIC CONDITIONS

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Introduction: Autologous chondrocytes harvested from articular cartilage are being used for the repair of focal cartilage defects. The procedure involves injury to the cartilage and alternative sources of stem cells for use in repair are being explored. Stem cells have been found in many tissue including bone marrow and the infrapatellar fat pad. Infrapatellar fat pad derived stem cells present a viable and easily accessible source of stem cells for the repair of cartilage defects and tissue engineering applications.

Hypoxia has been shown to improve chondrogenesis in stem cells derived from the bone marrow. We explore the hypothesis that this effect would also apply to stem cells derived from the infrapatellar fat pad.

Materials and methods: Cell aggregates from early passage stem cells isolated from the infrapatellar fat pad were placed in chondrogenic media for 14 days either in a normoxic (20% oxygen) or hypoxic (5% oxygen) environment. Gene expression analysis, DNA and glycosoaminoglycan assays and immunohistochemical studies were performed on the aggregates to assess chondrogenesis.

Results: Cells grown under hypoxic conditions showed significantly improved chondrogenesis as determined by relatively higher gene expression of proteoglycans, collagens and SOX genes. The cell aggregates also had a higher glycosoaminoglycan content and glycosoaminoglycan content per DNA. Immunohistochemical studies confirm enhanced production of collagen types I and II and aggrecan.

Discussion: These findings confirm the previously documented effects of hypoxic culture conditions on stem cells and extend the findings to include infrapatellar fat pad derived stem cells. Our findings suggest that oxygen tension has a role in regulating the function of stem cells as they undergo chondrogenesis. In culture these cells appear to function optimally in an atmosphere of reduced oxygen that more closely approximates documented in vivo oxygen tension. This has important implications in future tissue engineering applications of these cells.

OC44

DYNAMIC RELEASE OF GROWTH FACTORS FROM FRESH FROZEN CANCELLOUS BONE ALLOGRAFT

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Bone morphogenic proteins (BMPs) are members of the transforming growth factor beta (TGF-beta) family and play a central role in bone formation. These morphogens are known to be present in bone matrix however the characteristics of their release during the grafting process has not previously been defined. The aim of this study was to determine the release BMP-7 (osteogenic protein; OP-1) from cancellous allograft that occurs during impaction grafting for revision hip arthroplasty.

Forty, 10mm cubes of cancellous bone were accurately cut from the central region of 7 fresh frozen femoral heads. The cubes were centrifuged and washed to remove the marrow contents. The cubes were then individually washed and the fluid assayed for BMP-7 activity using a commercially available enzyme linked immuno-sorbent assay kit (Raybiotech Inc.). The cubes were then divided into 4 groups with samples from each femoral head in each group. Each

group was subjected to strain of either 20%, 40%, 60% or 80% using a material testing machine. The cubes were then individually washed again and the wash fluid analysed for BMP-7 activity.

BMP-7 activity was found to be present in all groups. Release of BMP-7 was found to increase with increasing strain. At 80% strain the mean concentration of BMP-7 released (830 pg/g) was 58% greater than that released at 60% strain (527 pg/g), 150% greater than the concentration at 40% strain (333 pg/g) and 476% greater than at 20% strain (144 pg/g). The differences between release at 80% and 40% strain and between 80% and 20% strain were statistically significant (p=0.036, p=0.002).

Activity of BMP-7 in fresh frozen cancellous allograft bone has not previously been demonstrated. This study shows that the freezing and storage of femoral heads allows some maintenance of biological activity. Furthermore we have shown that BMP-7 may be released in proportion to the strain applied to the bone. This confirms that the process of impaction of bone morsels during revision hip arthroplasty may release BMPs that could aid in the incorporation and remodelling of the allograft.

OC45

THE EFFECT OF LOCAL ADMINISTRATION OF PHENYTOIN ON FRACTURE HEALING: AN EXPERIMENTAL STUDY

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Background and objectives: The antiepileptic drug Phenytoin (Diphenyl hydantoin) has been documented to have a beneficial effect on wound healing. Its effect on fracture healing has been investigated to a much lesser extend.In this study we have combined histology, histomorphometry and radiology in analyzing the effect of phenytoin on fracture healing, following its local administration.

Methods: Twenty-four Wistar strain rats of 8-9 months age were assigned into two groups of 12 each, which had been matched for age, sex and weight. In one group, selected as the study group phenytoin 20 mg/kg was administered through a 24 gauge needle directly on to the fracture site every 72 hours, while in the controls an equivalent volume of normal saline was administered at the same interval. At 28 days radiographic and histological analysis was done.

Results: Radiographic and histological scoring across the control and test animals did not show any statistical difference. Histomorphometric analysis of the callus however showed that the total periosteal callus on either side of the central bridging callus was mineralized to a greater extend in the phenytoin group animals as compared to the control group animals (p= 0.011).

Conclusion: After analyzing our data, we concluded that phenytoin does have an influence in fracture healing, albeit small, which is primarily on the hard callus region. The hard callus region is the high oxygen tension region and the first region to differentiate. It appears that the effect of phenytoin is probably exerted at the early mesenchymal differentiation stage. However our preliminary work shows that the effect is small and it is not justifiable at this stage to advocate the use of phenytoin clinically to augment fracture healing.

OC46

SFA(PATH); A MODIFICATION OF THE SFA GRADING SYSTEM OF CHONDROPATHY IN OSTEOARTHRITIS FOR USE WITH PATHOLOGICAL SAMPLES

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Aim: Severity of knee osteoarthritis (OA) can be defined clinically, radiologically, or pathologically. The Système Française D'Arthroscopie (SFA) is a validated method of grading and scoring the severity of changes on the articular surface as observed through the arthroscope. We have validated a modification of the SFA system for use with digital photographs of pathological samples.

Material and Method: After Ethics Committee approval, both tibial plateaux and femoral condyles were collected from 84 patients undergoing total knee replacement or at post mortem. Extent and grading of cartilage changes were documented for the 4 compartments of each sample on a diagram using direct visualisation and probing (Pathological Scores). In addition, each sample was digitally photographed at standard magnification and illumination, archived, graded and scored (Photographic Scores). A second observer (AY) also graded and scored photographic images for 72 compartments of the first 18 cases.

Data analysis: Repeatability was measured as Repeatability Coefficients (Bland and Altman, Lancet 1986; 1; 307-10). 95% of the differences between 2 measurements of a case are expected to fall within the Repeatability Coefficient. Associations between compartments are expressed as Pearson correlation coefficients.

Results: For each of the 4 compartments studied, scores ranged from -2.2 to +717.8, representing the full range of possible scores. Allocation of scores to diagrams was highly repeatable (Repeatability Coefficient = 50). There was good agreement between Pathological and Photographic Scores (Repeatability Coefficient = 88). There was moderate agreement between Photographic Scores allocated by the 2 observers, with greatest agreement for low (<200) and high (>500) scores. Scores for each compartment correlated with scores for each of the other 3 compartments (R values 0.7 to 0.9, all $P < 0.005$).

Conclusion: Our modified SFA system permits scoring of OA severity using digital photographs of pathological samples. Our data support the view that OA affects the entire joint, and that a single compartment (e.g. medial tibial plateau) can be taken as broadly representative of the tibiofemoral joint as a whole.

OC47

THE SAGITTAL PLANE KINEMATICS OF A NEW UNICOMPARTMENTAL KNEE REPLACEMENT: A CADAVERIC STUDY OF THE 'DOMED' OXFORD LATERAL UNICOMPARTMENTAL KNEE REPLACEMENT

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Background: The Oxford unicompartmental knee replacement (UKR) use in the lateral compartment has been associated with a reduced flexion range and diminished femoral rollback. It is postulated that this may be due to a flat tibial tray replacing the domed anatomy of the lateral tibia, tightening the posterolateral flexion gap. A new design incorporating a domed tibial component and a biconcave meniscal bearing has been developed to increase; (i) the posterolateral flexion gap in deep knee flexion (ii) meniscal bearing movement and (iii) lateral femoral condyle (LFC) rollback. A cadaveric study was designed to test these three outcomes.

Methods: The sagittal plane kinematics of seven thawed fresh frozen cadaver specimens within an upright Oxford testing rig were assessed under three different conditions; (i) intact normal cadaver knee (ii) flat lateral Oxford UKR (iii) domed lateral Oxford UKR. Each condition was tested during three ranges of motion (ROM) and data recorded during a flexion or extension half cycle. Knee flexion angle (KFA) and displacement measures of the lateral collateral ligament (LCL), LFC rollback and anteroposterior meniscal bearing movement were performed throughout knee ROM using four [3 linear, 1 rotary] potentiometer devices. Potentiometer data was recorded as a voltage reading and subsequently converted to either a millimetre displacement or degree measure using a calibration formula. All data points were compared at 10 degree interpolations of KFA.

Results: The flexion half cycles demonstrated the flat Oxford lateral UKR achieved 80.7% of normal cadaveric LFC rollback. The domed Oxford lateral UKR achieved 108.8% of normal cadaveric LFC rollback. The ratio of LFC rollback in the domed to flat UKR's was 1.35 times (134.9%). Meniscal bearing movement in flexion demonstrated a domed to flat UKR ratio of 1.3 times (129.7%). Similar values were obtained for extension half cycles in favour of the domed Oxford lateral UKR. No significant differences were identified in LCL measures.

Conclusions: The domed Oxford lateral UKR implant allows for improved bearing movement and femoral rollback when compared to the flat Oxford lateral UKR. The sagittal plane kinematics of the domed Oxford lateral UKR as represented by femoral rollback values approximate those of the normal cadaver knee.

OC48

NINE YEARS FOLLOW UP AFTER THE FIRST AUTOLOGOUS HUMAN DISC REGENERATIONS AND REPLANTATIONS

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Massive disc herniations after surgical decompression develop secondary back pain due to important loss of nucleus material with instability. No earlier proposed method to restore disc function was biological.

Chondrocyte culturing allows living repair of lost disc tissue. The contained disc space appears particularly suitable for receiving those tissue cultures. Surprisingly disc replantations had not been attempted before.

In 1996 two women and one man (aged 38-55) underwent open resection of a massive disc herniation by hemi-laminotomy, twice at L5-S1, once at L4/5.

All the excised disc tissue was given to tissue culture in an identical protocol as in autologous chondrocyte transplantation (ACT) for articular cartilage repair. After sufficient cell multiplication (11.5-23 millions living cells in 750 µl) four weeks later the engineered autologous disc tissue was injected in suspension through a contra-lateral puncture under local anaesthesia.

In prospective follow up a simplified Oswestry Disability Index was recorded and functional radiographs and NMR were taken after one, three, six and nine years.

All three patients remained freed from radicular pain and vertebral symptoms over the whole follow up period. Two patients never had functional restrictions nor loss of working capacity (Oswestry 1 and 6), one after retirement at 5 years developed rheumatoid disease but is still unchanged at the lumbar spine. The third patient partially recovered from preoperative radiculopathy (slight loss of strength and sensitivity S1) but still works, with minor adaptations to his original professional activity (Oswestry 18).

Functional radiographs up to the last follow up didn't show vertebral instability. In all cases the replanted inter-vertebral disc space remained unchanged with minimal widening in one case.

In NMR all three discs had partial signal recovery. Twice during the first year a new outgrowth of disc tissue was observed at the site of the primary disc herniation opposite to the replanting injection, without any clinical correlation.

Three cases with massive lumbar disc herniations showed good clinical and large anatomical recovery persisting nine years after reimplantation of engineered autologous disc tissue. The encouraging results of this small pilot study led to further closely monitored clinical applications before wider propagation of biological disc repair surgery.

OP1

GENE, PROTEIN AND ELECTROPHYSIOLOGICAL EXPRESSION OF ION CHANNELS ON HUMAN TENOCYTES

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Background and objectives: Tenocytes change their structure, composition and mechanical properties to adapt to mechanical loading. Voltage gated and mechanosensitive ion channels may play a key role in human tenocytes to regulate some or all initial responses to mechanical stimulation. To date, there has been no direct investigation of ion channel expression by human tenocytes.

Methods: Human tenocytes were cultured from patellar tendon samples harvested from five patients undergoing routine total knee replacement surgery (mean age: 66 years; range 63-73 years). RT-PCR, Western Blotting and whole cell electrophysiological studies were performed to investigate the expression of different classes of ion channels within tenocytes.

Results: Human tenocytes express mRNA and protein encoding voltage operated calcium channel (VOCCs) sub-units (Ca alpha 1A, Ca alpha 1C, Ca alpha 1D, Ca alpha2 delta1) and the mechanosensitive tandem pore domain potassium channel (2PK+) TREK-1. They exhibit whole cell currents consistent with the functional expression of these channels. In addition, other ionic currents were detected within these tenocytes consistent with the expression of voltage gated potassium channels, voltage gated sodium channels, and other outwardly rectifying leak currents.

Discussion and conclusions: Human tendon cells show increased levels of intracellular calcium when stress is applied to them. One of the mechanisms by which this occurs is by the influx of extracellular calcium into the cell via ion channels. VOCCs and TREK channels have been implicated in mechanotransduction signalling pathways in numerous connective tissue cell types. This study suggests that these mechanisms may be present in human tenocytes. In addition, human tenocytes may express other channel currents. Ion channels may represent potential targets for the pharmacological management of chronic tendinopathies.

OP2

BONE MARROW-DERIVED MESENCHYMAL STEM CELLS CAN FUSE WITH TUMOUR CELLS IN VITRO AND HOME TO TUMOURS IN VIVO

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A major challenge for cancer gene therapy is to deliver the transgene(s) specifically into the tumour sites. Mesenchymal stem cells (MSCs) are capable of homing to many damaged tissues to participate in healing processes. Tumour development shares many common characteristics with wound healing, and tumours have been long regarded as wounds that never heal. We hypothesized that MSCs can home to tumour sites like they do in wound healing situations and also fuse with cancer cells once the get there.

MSCs were obtained from the bone marrows of GFP (Green Fluorescent Protein) transgenic rats, cultured and characterized as previously described. For in vitro studies, RIF-1 (mouse fibrosarcoma cells) cells were firstly labeled with a red fluorescent cell membrane dye (PKH26, Sigma) and were then co-cultured with an equal number of GFP-expressing MSCs for 72 h. Cell fusion was identified, by fluorescence microscopy, as the yellow fluorescence of the overlaid red (RIF-1) and green (GFP MSCs) fluorescence. This occurred within 72 h.

For in vivo study, RIF-1 cells were firstly implanted subcutaneously into C3H-HEN mice and then 1 million GFP MSCs were immediately injected through the tail vein in the same mouse. After 1, 3, 5 and 7 days, mice were killed and the tumours, brain, heart, kidney, liver, lung and spleen were excised for the analysis of GFP expression. Western blot analysis showed that GFP was expressed in lung as well as in the tumours at early time points (1-5 days), but by 7 days GFP expression was found only in the tumours and not in any other organs examined.

In conclusion, we have demonstrated that GFP-expressing rat MSCs can fuse with RIF1 tumour cells in vitro, home to tumour sites in vivo through the systemic circulation, and survive in the tumours for a prolonged period. The tumour microenvironment preferentially promotes the engraftment of MSCs compared with normal tissues, which makes MSCs a good candidate as a vehicle for delivering anti-cancer genes.

OP3

THE SIGNIFICANCE OF WIRE TENSION IN FINE-WIRE FIXATORS: AN ANALYSIS OF THE EFFECT OF WIRE TENSION ON THE STRESS DISTRIBUTION AT THE WIRE-BONE INTERFACE

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Fine-wire fixators are a powerful tool in the management of acute fractures, non-unions, mal-unions and limb lengthening. The tension in the wires is very important in achieving stiffness of the whole fixator construct and current guidelines suggests tensioning wires to 900-1275N. There is evidence that during long term use the tension in the wires can reduce significantly. The effects of a reduction in tension on the fixator stiffness has been well characterised however the effect on the stresses imparted on the bone at the interface with the wire remain unknown. The main aim of this study was to identify any relationship between wire tension and wire-bone interface pressure.

An experimental system utilizing artificial cancellous bone mounted on a tensioned 2mm wire and then loaded by a material testing machine was employed. Pressure sensitive film allowed determination of interface stresses. The experiment was repeated at wire tensions of 600, 900 and 1200N. All other variables were kept the same during testing.

The highest pressures were found closest to the wire. At 1200N the peak pressures were 6-8 MPa, at 900N of tension the pressures rose to 8-10 MPa and at 600N pressures up to 14 MPa were observed. Deeper in the bone the pressures observed at 600N tension were double that seen at 1200N.

This is the first characterisation of the relationship between interface pressure and wire tension in fine-wire fixators. At 1200N the highest pressures are less than the compressive yield strength of cancellous bone whereas at both 600 and 900N pressures are greater than the yield which may lead to loosening. We therefore conclude that a tension of 1200N be employed when applying fine-wire fixators and during long term treatments the tensions should be regularly monitored to prevent loss of tension.

OP4

ADENOSINE MODULATES MESENCHYMAL STEM CELL DIFFERENTIATION INTO OSTEOBLASTS

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Modulation of differentiation of mesenchymal stem cells (MSCs) to osteoblasts is an important subject area in relation to the development of new therapeutic strategies for bone disease. We have studied the role that adenosine and its receptors might play in this pathway, by using adenosine as the natural ligand, and a range of synthetic universal and specific adenosine receptor agonists and antagonists.

Adult rat femur MSCs were isolated and grown in culture in the presence of dexamethasone and ascorbic acid to induce them to differentiate along the osteoblast lineage. RT-PCR demonstrated that these cells expressed the 4 (A1, A2a, A2b, A3) adenosine receptor subtypes. When cells were incubated with a range of compounds (adenosine; NECA - universal agonist; CCPA, CGS21680, IB-MECA - specific A1, A2a, A3 agonists respectively) at a range of concentrations (0.1 mmolar - 10 nmolar) for up to 3 days, no significant effects were observed on cell number. Other than CGS21680, however, all of the compounds significantly increased alkaline phosphatase (ALP) activity in a concentration dependent manner. At 1 micromolar e.g. the increase was 49, 44, and 33% with NECA, IB-MECA and CCPA respectively when compared to controls. An increase of 27% was seen with 1 micromolar adenosine but only when the adenosine was replenished daily. When cells were induced to mineralise in culture over a period of 2 - 3 weeks, mineralisation was decreased by NECA and IB-MECA in a concentration dependent manner (e.g. more than 75% reduction with 1 micromolar NECA or IB-MECA when compared to controls). Adenosine had no effect, but

was not replenished daily in these experiments. In addition, using quantitative RT-PCR, NECA (10 micromolar) significantly decreased (by approximately 50%) the expression of connexin 43.

In summary, adenosine receptor agonists increase ALP activity, decrease mineralisation, and decrease the expression of connexin 43 in MSC derived osteoblasts. Modification of adenosine receptors in osteoblasts is thus a possible new therapeutic target in conditions when adenosine concentrations are known to be high (e.g. fracture, rheumatoid arthritis) and in bone disease generally.

OP5

A NOVEL APPROACH TO CREATING A BIO MIMETIC HYDROXYAPATITE COATING ON FINE SURFACE FEATURES TO ENHANCE OSSEOINTEGRATION OF EXTERNAL FIXATION SCREWS

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Background: As the understanding of bone repair mechanics has advanced the integrity of the bone pin interface has emerged as a key factor in determining the success of external fracture fixation. The benefits of using pins coated with Hydroxyapatite (HA) are well documented however the thickness of the conventional plasma spray coating precludes its use for modification of the surface of fine features in implants. Consequently new electro-chemical techniques for pre-coating implants with a 'biomimetic' HA layer using simulated body fluids (SBF) have been pioneered. In this study we test the hypothesis that varying the technique for deposition of HA by electrolysis of SBF alters the morphology of the HA surface which will modify the level of osseointegration. Method: Three alternative methods of HA coating the Barerre, Redepenning and Kumar techniques were compared. Tantalum coated stainless steel pins were coated then used to stabilise a mid-diaphyseal osteotomy in three sheep using an orthofix fixator for a period of ten weeks. Insertion and extraction torques were measured to calculate the pin performance index (PPI). Sections of the bones were then examined using scanning electron microscopy to determine the percentage of bone in contact with the pin surface and the percentage of new bone formation. Results: The different coating protocols resulted in different HA crystal morphologies. The extraction torque exceeded the insertion torque for both the Barerre and Redepenning methods and their PPI exceeds that of plasma spray coatings. The Redepenning technique was shown to perform significantly better than both the Barerre (p=0,001) and Kumar (p=0,001) techniques with 49.4% of the pin surface in contact with bone. These results were mirrored on analysis of new bone formation with the Redepenning technique showing 70.2% of new bone formation compared to the Barerre (55.4%) and Kumar (53.8%) methods. Conclusion: These results indicate that the Redepenning technique is the most effective for creating a bio mimetic HA coating in terms of bonding to bone and promoting new bone formation. This technique holds significant advantages over the conventional plasma spray technique for example the coating thickness can be easily controlled and additional proteins such as bone morphogenic proteins and antibiotics can be incorporated. It may therefore represent a new era in the use of HA coating.

OP6

MUTATIONS IN THE RANK SIGNAL PEPTIDE ALTER THE SUBCELLULAR LOCALISATION OF RANK AND PREVENT LIGAND-DEPENDENT ACTIVATION OF NF KAPPA BETA

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Receptor activator of nuclear factor kappa beta (RANK) is a transmembrane protein required for osteoclast formation and survival. Insertion mutations have previously been identified in the signal peptide of RANK in patients with familial expansile osteolysis (FEO), Expansile Skeletal Hyperphosphatasia (ESH) and severe/early onset Paget's Disease (PDB). It has been suggested that these mutations cause NFkappabeta activation, thereby stimulating osteoclast formation. To examine this further, we overexpressed FLAG-tagged constructs of these genes by transient transfection of 293 cells. Wildtype (WT) RANK was localised by immunofluorescence staining to plasma membranes as well as the golgi, whereas FEO-, ESH- and PDB-RANK were not detected at the plasma membrane, and appeared to accumulate in the ER, and in cytoplasmic vesicles. Ultrastructural studies showed that all three mutant forms of RANK, but not WT-RANK, induced large areas of intermediate filaments in transfected 293 cells, a feature commonly associated with overexpression of misfolded proteins and with inhibition, or overload, of the proteasome. In addition, expression of all transiently transfected RANK constructs caused similar levels of constitutive activation of NFkappabeta but only WT-RANK-transfected cells showed RANKL-dependent activation.

To examine the effect of the mutations under more physiological expression levels, we developed 293 cell lines stably expressing single copies of WT-, FEO-, ESH or PDB-RANK. By contrast to transiently transfected cells that overexpressed the RANK proteins, there was no constitutive activation of NFkappabeta in any of the stable cell lines. When the cells were stimulated for 60 minutes with RANKL, only cells stably expressing WT-RANK showed an increase in NFkappabeta activation above that of the untransfected 293 cell line, consistent with localisation of WT-RANK (but not the mutant forms) to the plasma membrane.

These results show that the FEO-, ESH- and PDB- mutations prevent the processing and delivery of RANK to the plasma membrane, and hence prevent RANKL-dependent activation of NFkappabeta. The mutant forms of RANK do not appear to cause constitutive activation of NFkappabeta except (like WT-RANK) when artificially overexpressed. The exact way in which these mutations affect osteoclast formation/function therefore remains to be determined.

OP7

BIOMIMETIC APPROACHES TOWARDS THE DESIGN AND SYNTHESIS OF TISSUE ENGINEERING SCAFFOLDS

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The ability to generate replacement human tissues on demand is a major clinical need. Indeed the paucity of techniques in reconstructive surgery and trauma emphasize the urgent requirement for alternative strategies for the formation of new tissues and organs. The idea of biomimesis is to abstract good design principles and optimizations from nature and incorporate them in the construction of synthetic materials and structures. Direct appropriation of natural inorganic skeletons is also biomimetic since their unique properties inform us on ways to generate functional, optimized scaffolds.

A number of well characterized natural skeletons were investigated as potential scaffolds for tissue regeneration using mesenchymal stem cell populations. Marine sponges, sea urchin skeletons and nacre were found to possess unique functional properties that supported human cell attachment, growth and proliferation and provided organic/ inorganic extracellular matrix analogues for guided tissue regeneration.

A good understanding of the processses involved in biomineralisation and the emergence of complex inorganic forms has inspired synthetic strategies for the formation of biological analogues (organised inorganic materials with biological form). We have developed two functional examples of biological structures generated using biomimetic materials chemistry with applications for human tissue regeneration. Mineralised biopolysaccharide microcapsules provided enclosed microenvironments with an appropriate physical structure and physiological milieu, for the support of the initial stages of tissue regeneration combined with a capacity to deliver human cells, plasmid DNA and controlled release of biological factors such as cytokines. Calcium carbonate porous microspheres analogous to microscopic coccolithophore shells provided a template for tissue formation and a mechanism for the delivery of DNA and functional biological factors. These biomimetic structures have considerable potential as scaffolds for skeletal repair and regeneration, particularly when combined with inductive and stimulatory biological factors (cytokines, morphogens, signal molecules) and plasmid DNA carrying with them chemical cues that modulate and direct permanent tissue formation complimentary with the host.

OP8

FUNCTIONAL AND ULTRASTRUCTURAL STUDIES OF OSTEOCLASTS FROM OSTEOPETROTIC PATIENTS

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Osteopetrosis is a clinical phenotype characterised by increased skeletal mass. In most cases the disease is caused by loss of function mutations in genes essential for acid production and secretion in osteoclasts, such as TCIRG1, CLCN7 and CAII. In some cases mutations in less-well characterised genes are responsible (OSTM1 and PLEKHM1) and in another group of patients no genetic defect has as yet been identified. We have started to analyse the functional phenotype of osteoclasts generated in vitro from patients with osteopetrosis to help understand the cellular basis of the disease. Osteoclasts were generated from peripheral blood mononuclear cells in the presence of RANKL and MCSF on dentine disks. Cells were examined by scanning and transmission electron microscopy (SEM and TEM) and by confocal microscopy. So far we have examined osteoclasts from 4 different genetic backgrounds: dominant osteopetrosis caused by a mutation in CLCN7, recessive osteopetrosis caused by mutations in CLCN7 or PLEKHM1 and a case with unknown genetic background. Osteoclasts developed in all cases and controls (unaffected siblings, or unrelated controls) and all cells expressed high levels of VNR. F-actin was seen in peripheral rings of podosomes in osteopetrotic osteoclasts, whereas proper sealing zones were seen in all resorbing cells. By SEM osteopetrotic osteoclasts were flat, well spread and had fewer membrane ruffles than the control resorbing cells. There was no sign of resorption in cultures from patients, whereas in all control cultures large, deep (>40 micron) resorption pits were seen made by osteoclasts with multiple microvilli. By TEM all non-resorbing osteoclasts contained large numbers of electron dense secretory granules, very similar to those seen in the osteopetrotic ia/ia rat where they have been found to contain TrAP. Resorbing cells contained many multivesicular bodies and other non-electron dense endosomal structures and very few secretory granules. These vesicular structures are currently being analysed using immuno-EM techniques. We conclude that in vitro generation of osteopetrotic osteoclasts reproduces the in vivo phenotype accurately and may help to identify further genetic causes. In addition, these studies offer the possibility to gain further insight in the vesicular transport routes within this highly specialised cell type.

OP9

CHANGES IN THE CHARACTERISTICS OF ARTICULAR CHONDROCYTES DURING OSTEOARTHRITIS

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Osteoarthritis (OA) is characterised by the progressive loss of the articular cartilage. This is accompanied by change in phenotype from cells expressing chondrocytic genes to cells, termed 'degradative' chondrocytes, that express aggrecanases and collagenases. To understand the cellular events involved, human articular cartilage was obtained from femoral heads after arthroplasty due to OA, fracture of the neck of femur (#NOF) due to osteoporosis, or from a 14 year old male (CDH). Samples were graded according to the new OARSI system (Osteoarthritis and Cartilage, 2006, 14:13-29) and paraffin sections were immunostained for c-Myc (marker of cellular activation), S100 (typically expressed in chondrocytes), Sox-9 (expressed in early-stage chondrocytes) and nucleostemin (a stem-cell marker). In addition, some specimen were incubated with fluorescent probes to identify metabolically activated cells (CellTracker green).

All chondrocytes, irrespective of OA grade, were immunopositive for S100, but there were differences in the other parameters. Cartilage from the 14-year old (OARSI grade =0) was characterized by no loss of proteoglycans (safranin-O) in the superficial zone and absence of c-Myc, Sox-9 and nucleostemin in all articular chondrocytes. In #NOF cartilage, proteoglycan loss was evident in the very superficial zone. Many chondrocytes in that zone showed bright green fluorescence with CellTracker-green and were c-Myc positive, consistent with cellular activation. Sox-9 and nucleostemin were absent. Mid-zone and deep zone chondrocytes showed no change. In low-grade OA samples (OARSI = 1-2), the zone of proteoglycan loss had increased, the CellTracker-green/c-Myc positive chondrocytes in that zone had divided to form clusters of 4-8 cells. Occasional cells were positive for nucleostemin. Mid-zone and deep zone chondrocytes still showed no change. In high-grade fibrillated OA cartilage (OARSI = 3-4) the superficial and mid zones had been eroded, leaving the deep zone at the surface. Chondrocytes were typically found in large clones, which were all immunopositive for c-Myc as well as for nucleostemin and Sox-9.

The results show that cellular activation starts near the surface and progresses to the deep zone. The presence of nucleostemin and Sox-9 suggests that de-differentiation may be involved in the phenotypic change from the chondrocytic to the degradative phenotype.

OP10

OSTEOBLASTS RESPOND TO MILD HEAT STRESS BY A CHANGE IN OSTEOPROTEGERIN (OPG) AND RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPA B LIGAND (RANKL) RATIO

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We have previously shown that molecular chaperones, also known as heat shock proteins (Hsps) and cell stress proteins, have a potent effect on bone remodeling(1;2). Hsps have evolved as intracellular protein-folding molecules essential in enabling cells to cope with environmental and physiological stress(3). Mild physiological stress such as change in pH or oxygen tension also has a potent effect on bone remodeling(4;5). We found that human Hsp60 induced bone resorption was inhibited by OPG(2). In this study we have investigated the effect of mild thermal stress on osteoblasts with reference to bone remodeling.

Osteoblastic cell line; MG63 cells were exposed to 33°C, 37°C and 42°C for 90 minutes, then incubated overnight at 37°C. We assayed for OPG and sRANKL and tumour necrosis factor alpha (TNF alpha) protein and mRNA and heat shock proteins 27, 60, 70 and 90.

Results showed that there was no expression of hsp 60 and 70, HSP 27 was slightly more up regulated at 33°C compared to 42°C, hsp 90 was expressed at all temperatures studied. There was no change in TNF alpha levels. The expression of RANKL was significantly reduced at 42°C whereas there was no difference in the OPG levels. At 33°C OPG levels were significantly raised, and the RANKL levels were not affected.

These results show osteoblasts respond differently to hyper- and hypothermia, mild thermal stress suppresses RANKL synthesis at 42°C, with no effect on OPG in contrast at 33°C, OPG synthesis is suppressed with no effect on RANKL synthesis. In both cases altering the OPG/RANKL ratio, the balance between RANKL and OPG is critical for modulation of bone remodeling and in both cases would lead to inhibition of bone resorption

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OP11

EXPERIMENTALLY VALIDATED FINITE ELEMENT MODEL OF A HUMAN TIBIA WITH A UNICOMPARTMENTAL KNEE REPLACEMENT

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Finite element (FE) analysis is widely used to calculate stresses and strains within human bone in order to improve implant designs. Although validated FE models of the human femur have been created (Lengsfeld et al., 1998), no equivalent yet exists for the tibia. The aim of this study was to create such an FE model, both with and without the tibial component of a knee replacement, and to validate it against experimental results.

A set of reference axes was marked on a cleaned, fresh frozen cadaveric human tibia. Seventeen triaxial stacked strain rosettes were attached along the bone, which was then subjected to nine axial loading conditions, two four-point bending loading conditions, and a torsional loading condition using a materials testing machine (MTS 858). Deflections and strain readings were recorded. Axial loading was repeated after implantation of a knee replacement (medial tibial component, Biomet Oxford Unicompartmental Phase 3). The intact tibia was CT scanned (GE HiSpeed CT/i) and the images used to create a 3D FE mesh. The CT data was also used to map 600 transversely isotropic material properties (Rho, 1996) to individual elements. All experiments were simulated on the FE model. Measured principal strains and displacements were compared to their corresponding FE values using regression analysis.

Experimental results were repeatable (mean coefficients of variation for intact and implanted tibia, 5.3% and 3.9%). They correlated well with those of the FE analysis (R squared = 0.98, 0.97, 0.97, and 0.99 for axial (intact), axial (implanted), bending, torsional loading). For each of the load cases the intersects of the regression lines were small in comparison to the maximum measured strains (<1.5%). While the model was more rigid than the bone under torsional loading (slope =0.92), the opposite was true for axial (slope = 1.14 (intact) 1.24 (implanted)) and bending (slope = 1.06) loads. This is probably due to a discrepancy in the material properties of the model.

OP12

LEFT-HANDEDNESS IN WOMEN IS ASSOCIATED WITH A LOWER RISK OF FRACTURE BEFORE THE AGE OF 40

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Other studies have observed that left-handedness is associated with earlier menarche. Earlier menarche has also been linked to a lower risk of postmenopausal fracture. Our objective was to investigate whether hand dominance influences fracture risk in the pre-menopausal years.

Our population-based cohort (the Sheffield part of the OPUS study) consisted of 600 women: 99 were age 20-40 years and 501 were 55-80 years. We collected data including dominant hand, date of birth, age of menarche and prior fractures with ages. Bone mineral density (BMD) was measured at the lumbar spine and hip.

It was common practice in earlier years to force right-handedness in children, and women born more than 70 years before the start of the study were found to be less likely to be left-handed than the rest of the cohort (5.7% v 10.8%, p=0.05). These subjects and one ambidextrous subject were excluded from the analysis because of uncertainty about their natural dominance.

In the remaining group (n=424), we confirmed that left-handedness was associated with earlier menarche, by a difference of 0.74 years (p=0.005). However, age of menarche was not associated with prior fracture before age 40. Such fractures had been sustained by 129 subjects. 122 out of 378 who were right-handed, but only 7 out of 46 left-handers had had a fracture before age 40 (p=0.018). By logistic regression, the odds ratio for a right-handed woman of having a fracture when young was 2.66 (95%CI 1.15, 6.11). Of the women without early fractures (n=295), dominance was not associated with prior later fracture.

In the younger group, who were at approximately peak bone mass, there was no relationship between dominance or age of menarche and BMD at the spine or any region of the hip, nor was there any consistent trend for either variable.

We conclude that left-handedness is associated with earlier menarche and with a lower risk of fracture before the age of 40. The association between dominance and early fractures is independent of age of menarche and BMD at any site.

CC1

COMPARISON OF THE USE OF HUMERUS INTRAMEDULLARY NAIL AND DYNAMIC COMPRESSION PLATE FOR THE MANAGEMENT OF DIAPHYSEAL FRACTURES OF HUMERUS: A PROSPECTIVE RANDOMISED STUDY

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The aim of this study was to compare the results of humerus intramedullary nail(IMN) and dynamic compression plate DCP) for the management of diaphyseal fractures of humerus.

Material & Methods- 47 patients with diaphyseal fracture of shaft humerus were randomised prospectively and treated by open reduction and internal fixation with IMN or DCP.The criteria for inclusion was Grade 1,2a compound fractures, polytrauma, early failure of conservative treatment, unstable fracture . The patient with pathological fracture, Grade 3 open fracture, refracture, old neglected fracture of humerus were excluded from the study. 23 patients underwent internal fixation by IMN and 24 by DCP. Reamed antegrade nailing was done in all cases. DCP was done through an anterolateral or posterior approach.

Results -The outcome was assessed in terms of functional outcome and the incidence of complications. Functional outcome was assessed using the Americans Shoulder and Elbow Surgeons Score (ASES). On comparing the results, there was no significant difference in ASES score between the two groups. The rate of complications was found to be higher in patients treated with IMN than with DCP. The complications that were encountered with IMN were non union, shortening of the arm, impingement of the shoulder, implant failure. The rate of secondary surgery was also found to be significantly higher with IMN.

Conclusion - There is sufficient evidence to suggest that DCP still remains to be the operative treatment of choice for diaphyseal fractures of humerus. IMN may be indicated only in specific situations like segmental fractures, pathological fratures though this study did not aim to look into that aspect.

CC2

A BIOMECHANICAL STUDY COMPARING 6.5MM CANCELLOUS SCREWS AND 3.5MM CORTICAL SCREWS FOR DEPRESSED TIBIAL PLATEAU FRACTURES

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Introduction and aims: There is a recent trend of using a raft of small diameter 3.5 mm cortical screws instead of the large diameter 6.5mm screws in depressed tibial plateau fractures (Schatzker type 3). Our aim was to compare the biomechanical properties of these two constructs in the normal and osteoporotic sawbone model.

Methods: 10 sawbone (solid rigid polyurethane foam) blocks with a density simulating that of an osteoporotic bone and 10 blocks of a density simulating normal bone were obtained. A Schatzker type 3 fracture was created in each block. The fracture fragments were then elevated and supported using 2, 6.5mm cancellous screws in 10 blocks and 4, 3.5mm cortical screws in the remaining.

The models were loaded to failure using a Lloyd's machine. A displacement (depression) of 5mm was taken to be the point of failure. A load displacement curve was plotted using Nexygen software and the force needed to cause a depression of 5mm was calculated in each block. Mann Whitney U test was used for statistical analysis.

Results: Osteoporotic model

The mean force needed to produce a depression of 5mm was 700.8N with the 4-screw construct and 512.4N with the 2 screw construct. This difference was statistically significant (p=0.007).

Non-osteoporotic model

The mean force requires to produce the same depression was 1878.2N with the 2-screw construct and 1938.2N with the 4 screw construct. The difference was not statistically significant (p=0.42).

An increased fragmentation of the sawbone fragments was noticed with the 2-screw construct but not with the 4-screw construct.

Conclusion: A raft of 4, 3.5 mm cortical screws is biomechanically stronger than 2, 6.5mm cancellous screws in resisting axial compression in osteoporotic bone.

CC3

AUTOSOMAL DOMINANT CARPAL TARSAL OSTEOLYSIS: RESULTS OF TREATMENT WITH PAMIDRONATE

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This 3 year old girl fractured her right distal radius and ulna in a fall from a bike. She was treated with a cast but continued to complain of pain in her right wrist. Her father was known to have osteolysis. A further radiograph confirmed carpal osteolysis. The left wrist appeared normal.

The patient has mild dysmorphis features with a high forehead and hair line. The nasal bridge is prominent and she has a slightly small jaw. She has suffered increasing disability with pain in her wrists and ankles. Radiographs and MRI demonstrated progressive destruction of carpal and tarsal bones. Treatment with pamidronate for 6 months increased bone density but did not improve symptoms or prevent progression.

Hereditary osteolysis of the carpal and tarsal bones in most frequently an autosomal dominant condition. There are characteristic facial features as present in our patient and some individuals develop renal failure. A variety of eponymous syndromes have been described but probably represent a spectrum of severity of the same condition. The aetiology is unknown but a defect in the matrix metalloproteinase 2 gene has recently been described in a group of Saudi Arabian patients with a recessive pattern of inheritance. No treatment has been shown to be effective.

CC4

OSTEOGENIC OSTEOMALACIA ASSOCIATED WITH AN ETHMOIDAL SINUS TUMOUR

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We report a case of oncogenic osteomalacia associated with an ethmoidal phosphaturic mesenchymal tumour. This is an acquired paraneoplastic syndrome of phosphate wasting.

An 80 year old lady presented following a simple fall with bilateral fractured neck of femurs, insufficiency fractures of all four pubic rami and several old rib fractures. She was previously well with mild asthma and congestive heart failure only. Laboratory investigations showed a serum phosphate 0.29 mmol/l, PTH 14.8pmol/l, ALP 740 IU/l, normal calcium and vitamin D levels and normal serum electrophoresis. A diagnosis of osteogenic osteomalacia was made given the history of osteopenia, multiple low trauma fractures and phosphate wasting. This was confirmed by serum fibroblast growth factor (FGF-23) 8x normal levels. A subsequent whole body T2 STIR MRI revealed a hyperintense mass in her left ethmoid sinus. On resection this was shown to be a benign phosphaturic mesenchymal tumour secreting FGF 23. She is currently well, having stopped both phosphate and calcitriol supplementation with no signs of tumour recurrence.

Osteogenic osteomalacia is a rare disorder that has contributed to an increased understanding of normal phosphate homeostasis. Tumours that cause oncogenic osteomalacia are often small and slow growing and hence can be difficult to detect. Phosphaturic mesenchymal tumours account for 70-80% of tumours associated with oncogenic osteomalacacia, they most commonly occur in the appendicular skeleton but can occur anywhere, they are usually benign. FGF 23 has been shown to be abundantly expressed in most but not all tumours. It has also been shown to be the defective gene in autosomal dominant hypophosphatemic rickets. Circulating FGF 23 is detectable in normal human serum. Transgenic mice that over express FGF 23 clinically mimic this syndrome, conversely deficient mice have hyperphosphatemia, elevated calcitriol and hypercalcaemia. FGF 23 has been shown to exert its activity at the proximal tubule resulting in the inhibition of phosphate reabsorption and the downregulation of 25 hydroxyvitamin D-1-alpha-hydroxylase with decreased circulating levels of calcitriol, this dual action results in hypophosphatemia, poor mineralisation and increased fractures.

P1

TRANSLATION FROM LABORATORY TO THEATRE: AUGMENTATION OF IMPACTED ALLOGRAFT WITH HUMAN BONE MARROW STROMAL CELLS

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Introduction: Bone is unique with a vast potential for regeneration from cells with stem cell characteristics. With an increasing aging population, clinical imperatives to augment and facilitate tissue repair have highlighted the therapeutic potential of harnessing mesenchymal populations from bone. We describe laboratory and clinical findings from two clinical cases, where different proximal femoral conditions (AVN, bone cyst) were treated with impacted allograft augmented with marrow-derived allogeneic progenitor cells.

Methods: Marrow was aspirated from the posterior superior iliac crest and seeded onto prepared washed morsellised allograft. The seeded graft was left for 40 minutes to allow adherence of the marrow-derived osteoprogenitor cells prior to impaction into the defect. Samples of the impacted graft were taken for in-vitro analysis of cell viability, histology and biochemical analysis of cell number and osteogenic enzyme activity. The total force imparted during impaction was calculated using a load cell, with three independent surgeons performing a laboratory simulation of the impaction technique.

Results: Both patients made a rapid clinical recovery after an overnight stay. Imaging confirmed filling of the defects with increased density on plain radiographs suggesting good impaction of the graft composite. Immunohistochemical staining of graft samples demonstrated that a living composite graft with osteogenic activity had been introduced into the defects as evidenced by cell tracker green viability and alkaline phosphatase (osteogenic marker) expression and specific activity. The average peak forces during impaction were 0.7kN corresponding to average peak stresses within the graft of 8.3MPa. Similar forces were seen between operators.

Discussion: Replacement of bone loss remains a major challenge in orthopaedic practice. Although allograft remains the gold standard where large volumes preclude autograft, allograft has little osteoinductive potential. We demonstrate that marrow-derived cells can adhere to highly washed morsellised allograft, survive the impaction process, and are of the osteoblastic phenotype creating a living composite. Thus we conclude, impacted allograft seeded with autologous marrow cells allows the delivery of a biologically active scaffold for the treatment of bone deficiency. In addition this is a novel straightforward technique, surgeon independent and with applications in a number of orthopaedic scenarios.

P2

THE EFFECT OF OSTEOPOROSIS ON BONE MINERAL DENSITY AND FRACTURE REPAIR IN A RAT FEMORAL FRACTURE MODEL

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Background & Objectives: Osteoporosis is one of the most prevalent bone diseases worldwide with fractures its major clinical consequence. Studies on the effect of osteoporosis on fracture repair are contradictory and although it might be expected for fracture repair to be delayed in osteoporotic individuals, a definitive answer still eludes us. Subsequently, the aim of this study was to attempt to clarify any such effect.

Methods: Osteoporosis was induced in 53 female Sprague-Dawley rats by ovariectomy (OVX) at 3 months. A femoral fracture was produced in these animals 12 weeks later {OVX+Fracture group (OVX+F)}. A control group received the fracture only group (F) at 6 months. The fracture consisted of an open osteotomy held with a unilateral external fixator. Outcome measures include histology, motion detector analysis, pQCT, biomechanical strength testing (BST) and digital radiography. Digital radiographs were taken at time of OVX, fracture (confirming satisfactory reduction) and sacrifice from which relative bone density (BMD) measurements were calculated.

Results: OVX+F animals were significantly heavier than F animals at fracture and sacrifice ($p<0.001$ for both) and moved significantly less in days 1-4 ($p=0.032$) and 5-9 ($p=0.020$) post-fracture. Relative BMD measured in distal femur at fracture and sacrifice was significantly greater in F group ($p<0.001$ for both). Furthermore, there was a significant decrease in relative BMD from fracture to sacrifice in OVX+F group ($p<0.001$). pQCT showed a significantly greater total BMD {contralateral ($p=0.021$) and fractured femora ($p<0.001$)} and trabecular BMD ($p<0.001$ both limbs) in the distal femur of the F group. Histologically, no statistical differences were found, however, the F group generally displayed the most advanced repair. In the contralateral limb, the F group had significantly greater load to failure at 6 ($p=0.026$) and 8 ($p=0.042$) weeks and was significantly stiffer at 8 weeks ($p=0.050$). In the fractured leg, stiffness was significantly greater in the F group at 8 weeks ($p=0.001$).

Conclusion: OVX was linked to increased body weight, decreased motion, decreased BMD (with particular loss in trabecular BMD), and reduced mechanical properties. OVX did not have a significant effect on fracture healing and although there was no reduction in BMD at the fracture site, histology and reduced stiffness suggest it was delayed.

P3

EFFECTS OF TRANSFECTION OF RECOMBINED RAT TRANSFORMING GROWTH FACTOR BETA-1 AND RECOMBINED RAT INSULIN-LIKE GROWTH FACTOR-1 ON RABBIT CHONDROCYTES EX VIVO

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Studies have demonstrated that use of peptides including bone morphogenetic proteins, fibroblast growth factors, insulin-like growth factor (IGF), and transforming growth factor-beta (TGF-beta), may be pivotal in promoting chondrogenesis and matrix development. As a prelude to studies, it is necessary to determine which gene or combination of genes gives the best result to improve proliferation of chondrocytes and synthesis of extracellar matrix. We investigate the effect of transfection of recombined rat TGF-beta1 and recombined rat IGF-1 on rabbit chondrocytes ex vivo.

Chondrocytes were isolated from articular cartilage of knee joint of mature New Zealand White rabbits. Cells were seeded at a density of 1_105 cells/ml into 6-well plates. Monolayer cultures were infected respectively with recombinant rat gene pcDNA3+TGF-beta 1, pAT153+IGF-1 and lac Z reporter gene by using lipofectamine, and were co-transfected by pcDNA3+TGF-beta 1, pAT153+IGF-1. The control group remained uninfected. To determine whether the genes transcript were translated and the gene products were released, the synthesis of TGF-beta 1, IGF-1,and type II collagen were measured by in situ hybridization, immunohistochemistry and immunofluoroscopy. The proliferation of chondrocytes was detected by flow cytometer and 3H-TdR radiolabeling.

The expression of TGF-beta1,IGF-1 and type II collagen in recombinant rat gene transfection groups was high beyond control levels and the lac Z gene levels ($P<0.05$).The co-transfection elevated these factors synthesis beyond the levels of single gene transfection ($P<0.05$). In pcDNA3 +TGF-beta1 transfection group, the level of TGF-beta1 and type II collagen were higher than the levels of pAT153+IGF-1 group ($P<0.05$), while the content of IGF-1 has no significant difference with pAT153+IGF-1 group. By using flow cytometer, the chondrocytes ratio of S stage in pcDNA3+TGF-beta 1 group, pAT153+IGF-1 group and co-transfection group was 33.4%,28.7% and 40.1% respectively, which was higher than 5.6% and 4.8% of the control group and the lac Z gene group ($P<0.05$). The 3H-TdR radiolabeling detection also indicated that the recombinant rat gene transfection groups improved the chondrocytes proliferation, and co-transfection group has the best effect.

The data presented support that transfection of genes of TGF-beta1 and IGF-1 into chondrocytes ex vivo can greatly increase cell proliferation and matrix synthesis, and the co-transfection can provoke more increase in the synthesis of TGF-beta1, IGF-1 and type II collagen, which encourages the further research of gene potential therapeutic use for osteoarthritis.

P4

REPAIRING RABBIT ARTICULAR CARTILAGE DEFECTS WITH AUTOLOGOUS BONE MARROW MESENCHYMAL STEM CELLS

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Once damaged, articular cartilage has limited capacity for self-repair due to their avascular and acellular nature. Tissue engineering approaches using cultured chondrocytes and biomaterials as scaffoldings hold promises for repairing cartilage defects. However, the source of articular chondrocytes is limited and the chondrocytes may de-differentiate when cultured for a prolonged period. Bone marrow derived mesenchymal stem cells (BMSCs) have multi-differentiation potentials and autologous BMSCs are easy to obtain and culture with no/little immunological reaction when re-implanted.

24 NZW rabbits were used. Rabbit autologous BMSCs were obtained through marrow aspirations and expanded in culture under the chondrogenic induction media (DMEM, 10% FCS, plus 10ng/ml TGF-ß1) for 3 weeks. A full-thickness articular cartilage defect (3 mm in diameter and 3 mm in depth) was created on both medial condyles in the rabbit. For experimental group (16 joints), the defects were filled immediately with alginate capsules containing autologous chondrogenic cells (8.5 x 104); for the control groups, the defects were filled with either alginate capsules alone (16 joints) or left untreated (16 joints). All the animals were terminated at 6 and 12 weeks after surgery and the cartilage samples were harvested for histology, immunochemistry and in situ hybridization examinations.

For histology, in the experimental group the defects were filled with immature hyaline-like cartilaginous tissues at 6 weeks; by 12 weeks the newly formed cartilage showing signs of remodeling and integrating into the surrounding articular cartilage. The expression of type II collagen in the newly formed cartilaginous tissues was confirmed by immunohistochemistry and by in situ hybridization methods. In the control groups, the defects were mainly filled with fibrous tissues in all the animals at the two time points examined. We have used Wakitani cartilage grading system for semi-quantitative histological evaluation. Significant lower scores (with superior histology) were found in the experimental group comparing to the two control groups.

Our results confirmed that full-thickness articular cartilage defects can be repaired by chondrogenically differentiated autologous BMSCs seeded into alginate capsules. Further studies are ongoing to explore the long term outcome of this treatment approach as well as using new scaffolds for cartilage tissue engineering.

P5

A NEW HIP SIMULATOR FOR IN-VITRO FATIGUE TESTING OF IMPLANTED ACETABULA

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A new hip simulator has been developed at the University of Portsmouth and manufactured at Simulation Solutions, Ltd. (UK) for the purpose of fatigue testing of implanted acetabula. Although hip simulators for in vitro wear testing of prosthetic materials in total hip arthroplasty (THA) have been available for many years, similar equipment has yet to appear for endurance testing of fixations in cemented THA, despite of considerable evidence of late aseptic loosening as one of the most singnificant failure mechanisms in acetabular replacements[1].

In this study, a new four-station hip simulator designed for in vitro fatigue testing of implanted acetabula is described. The four-station machine has spacious test cells that can accommodate full hemi-pelvic bones with implants. The machine was designed to simulate the direction and the magnitude of the hip contact force relative to the acetabular cup coordinate system, as reported by Bergmann et al. [2], under typical physiological loading conditions, including stair climbing as well as walking. The controls were designed as such that each station may operate independently with a loading waveform that is fully programmable. The motions were achieved through two encoded servomotors suitably connected to gearboxes; while the loading was realised through a close-looped pneumatic system. The motions and the resultant hip contact force of the new hip simulator were evaluated, and found to be satisfactory in reproducing the typical physiological loading waveforms including normal walking, ascending and descending stairs.

Experiments have been carried out using third generation composite bones (Pacific Research Laboratories, Inc.) and bovine bones. Both hip simulator and conventional fatigue testing were carried out. The implanted acetabula were CT scanned periodically to monitor the damage development in the fixation. Preliminary results seem to suggest that both magnitude and direction of the hip contact force influence the integrity of the fixation, and failures appear to occur earlier in samples tested using the hip simulator. The predominant failure mechanism appears to be interfacial fracture, consistent with clinical observation of radiolucent lines and bone-cement interfacial failure.

P6

Abstract withdrawn

P7

THE EFFECT OF ATORVASTATIN TREATMENT ON BONE MINERAL DENSITY AND FRACTURE REPAIR IN A RAT FEMORAL FRACTURE MODEL

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Background & Objectives: Statins have been shown to stimulate bone formation in vivo and in vitro in rodent models1 generating interest in the possibility that they may be useful therapeutic agents for osteoporosis. The major clinical consequence of osteoporosis are fractures that occur and although there is no firm evidence, there is a perceived associated delay in fracture repair. We examined the influence of atorvastatin on fracture repair in an ovariectomised rat fracture model.

Methods: 126 Sprague-Dawley rats had an ovariectomy (OVX) at three months and a femoral fracture (F) at six months. The fracture consisted of an open osteotomy held with an external fixator. All animals were randomly assigned into groups 1. OVX+F and early atorvastatin; 2. OVX+F and late atorvastatin; 3. OVX+F. Atorvastatin (5mg/kg) was given daily by oral gavage for three months in-group 1 between OVX and fracture and from time of fracture to sacrifice in-group 2. Outcome measures were histology, peripheral quantitative computed tomography (pQCT), biomechanical strength testing (BST) and digital radiography. Digital radiographs were taken at time of OVX, fracture (confirming satisfactory reduction) and sacrifice from which relative bone density (BMD) measurements were calculated.

Results: Non-statin treated animals moved significantly more in 4 days post-fracture (p=0.015), had significantly more relative (p=0.037) and total BMD (distal femur) than statin treated (p=0.040, early and p=0.036, late treatment). Total BMD at the fracture site was also significantly greater in the OVX+F than the late statin group (p=0.047) while in the adjacent site of the contralateral limb, the early statin group had significantly more (p=0.018) than the late statin group. However no differences were found between the early statin and OVX+F groups. Histologically, the rate of repair increased significantly in early statin (p=0.013) and OVX+F (p=0.011) groups. BST data showed no significant difference in stiffness at six or eight weeks.

Conclusion: Fractures healed in all three groups. Statins did not prevent OVX induced bone loss. Initial evidence suggests that early statin treatment may have a positive effect on early fracture, as shown by x-ray analysis and histology, however this effect was lost by week 8. Overall the evidence suggests that atorvastatin may have impaired fracture repair, particularly with late administration (relative BMD and pQCT results).

P8

TREATMENT OF VANCOUVER TYPE B-2 PERIPROSTHETIC FRACTURE OF THE FEMUR USING AN EXTENSIVELY HYDROXYAPATITE COATED REVISION STEM

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Purpose: Periprosthetic fractures around hip prostheses are difficult problems because these fractures range from the very simple (requiring no surgical intervention) to the complex (requiring major surgery). This paper evaluates the primary stability and restoration

of femoral bone stock following treatment of Vancouver type B-2 periprosthetic fracture of the femur using an extensively hydroxy-apatite coated revision stem implant.

Methods: We have prospectively reviewed 9 cases with B-2 periprosthetic fracture operated at our centre between 1996 to 2001. Of the nine patients, 4 were male and 5 female. The mean age was 76.7 years (50-92). All patients were treated by femoral revision using an extensively hydroxy-apatite coated titanium revision femoral stem (Restoration HA, Stryker, Rutherford,NJ). Fixation was augmented with a combination of cerclage cables and onlay cortical strut allografts.

Results: There has been no loss to follow-up. 1 patient died, but at most recent follow-up the fracture had united with radiological evidence of bone on-growth to the stem. Mean follow up in the rest of the cohort was 3.3 years. There was radiological evidence of fracture union in all patients. Mean subsidence of the stem was 0.22mm. At most recent follow-up the mean Harris Hip Score had improved to 77.2 (63-93). Favourable bone remodelling was observed in all patients with no evidence of stress shielding so far. At most recent follow-up there have been no cases of mechanical failure, deep infection or dislocations. No patient is awaiting further revision.

Conclusion and Significance: Te Restoration HA stem has produced excellent clinical results in our study. We have observed no intra operative fracture and low postoperative complication rate. We are extremely encouraged by the observed femoral remodelling. There has been no case of mechanical failure as yet and there is no reason to expect, once union and on-growth have occurred, that loosening will be a problem. In treating this challenging and increasingly common complication of total hip replacement, femoral revision using an extensively HA coated revision femoral component offers a reliable method of femoral fixation leading to successful fracture healing and early return to function.

P9

RELEVANCE OF BONE DENSITY MEASUREMENTS INCLUDING THE FOREARM IN PATIENTS AWAITING JOINT REPLACEMENT FOR HIP AND KNEE OSTEOARTHRITIS

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This study aimed to determine the prevalence of osteoporosis in patients awaiting hip and knee replacement for osteoarthritis and to review them two years later to determine the changes in bone density following joint replacement.

Patients aged between 65 and 80 years awaiting total hip or knee replacement were invited to participate. Lumbar spine, bilateral femoral and forearm bone mineral density (BMD) measurements were obtained using dual energy x-ray absorptiometry. BMD values were standardised using previously published T-scores and Z-scores. To assess clinical status, patients completed a questionnaire including the Western Ontario and McMaster University OA Index (WOMAC). All measurements were repeated at two-years.

Participants included 199 patients (84 hips and 115 knees) with a mean age of 72 years (SD 4.0) and were predominantly female (hips 67%, knees 50%). At baseline 46/199 (23%) patients (39 females) had evidence of osteoporosis (WHO definition) at one or more sites with the highest prevalence at the forearm (14%). At two-years 144 patients attended for review with 128 having undergone hip (56) or knee (72) replacement. At this review 39/144 (27%) patients (33 females) had evidence of osteoporosis at one or more sites with the highest prevalence at the forearm (22%). The greatest bone loss occurred at the forearm with median BMD change of minus 4% for females (25th percentile minus 7.3%, 75th percentile minus 1.9%) and minus 2.9% for males (25th percentile minus 4.6%, 75th percentile minus 1.1%). There was a significant difference in WOMAC Pain scores at follow-up between the osteoporotic and non-osteoporotic knee patients (67 versus 81, p=0.002) indicating that osteoporotic patients had greater knee pain.

We have identified the forearm as not only the site with the highest prevalence of osteoporosis but also the greatest bone loss at follow-up. Further evaluation of forearm bone density measurements in the preoperative assessment and follow-up of patients awaiting joint replacement for hip and knee OA is required. Larger studies are needed to confirm our finding that the presence of osteoporosis is predictive of worse patient-reported outcomes of knee replacement.

P10

RELEASE OF GROWTH FACTORS FOLLOWING FRACTURE FIXATION

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Purpose: Growth factors are released and circulate in peripheral blood after fracture. The purpose of this study was to characterize the early systemic release of several growth factors following accidental fractures and surgery.

Methods: 14 patients (8 male and 6 female) suffering from lower limb long bone fractures were prospectively included in the study. The mean age was 34 years (range 18-61). In all patients the time from fracture occurrence till operation was less than 24 hours. Peripheral blood samples were collected on patients' admission, induction, and postoperatively at 1, 3 and 5 days. Serum was extracted and using Elisa colorimetric assays the concentration of Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Insulin-like Growth Factor I (IGF-1) and Transforming Growth Factor beta 1 (TGF-b1) was measured.

Results: From fracture occurrence till induction for surgery a substantial decreased was observed (VEGF concentration was decreased by 189%, PDGF was decreased by 363%, TGF-b1 was decreased by 247 % and IGF-1 was decreased only by 25%. Surgery itself decreased VEGF peripheral levels by a further 50% and PDGF by 40 % while IGF and TGF-b1 levels remained unchanged. During the first post-operative day the levels of VEGF were increased by 82%, TGF-b1 and IGF-1 remained constant and PDGF was further decreased by 20%. Between the 1st and 3rd postoperative days VEGF was increased by 132%, PDGF by 220% and TGF-b1 by 230 %. During this period, IGF-1 was decreased by 20 %. Finally, during the 3rd to 5th postoperative day, the levels of all growth factors continue to increase.

Conclusion: This study illustrates the early pattern of release of 4 growth factors following fractures and surgery. A substantial decreased during the first 24 hours was noted but thereafter an upward trend was observed. This data provide insight into the levels and kinetics of growth factors before and after surgery of fractures.

P11

HOW TO BEST IMMOBILISE PARTIALLY WEIGHT BEARING EXTENSOR MECHANISM INJURIES: A GAIT ANALYSIS STUDY

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Patella and extensor mechanism injuries are common injuries and are generally managed with some degree of immobilisation and partial weight bearing to facilitate healing. The aim of this project was to determine the type of immobilisation or splintage during partial weight bearing that results in minimal forces acting through the extensor mechanism.

Gait analysis studies were performed on eight healthy male subjects mobilising partially weight bearing. Measurements were taken for six types of immobilisation: locked at 0, 10, 20, 30 degrees and unlocked in an orthotic knee brace, and without a brace. The ground reaction force, knee joint angle and the knee flexion moment were measured using Qualisys Track Manager and Visual 3D Software. The extensor mechanism moment and the extensor mechanism force were calculated using static equilibrium equations and documented data. A one-way analysis of variance statistical test was performed to determine the statistical significance of the differences between the six types of immobilisation.

There was a direct relationship between the knee flexion angle and the extensor mechanism force. The extensor mechanism force at 0 degrees of immobilisation was significantly lower than that for 20 and 30 degrees ($p<0.05$). The increase in the extensor mechanism moment arm with increasing knee flexion was not sufficient to offset the increase in the extensor mechanism force caused by the increase in the knee flexion moment. The results also showed that the knee flexion angle does not always correspond with the angle set at the knee brace; however they did exhibit a direct relationship.

These results have important implications for the management of patients with patella and extensor mechanism injuries. The results

suggest that improvements in knee brace design to allow 0 degrees of knee flexion, rather than the 10 degrees as seen in this study, are likely to result in significantly reduced extensor mechanism tensile forces.

P12

ß-DYSTROGLYCAN AND MATRIX METALLOPROTEINASES - ALTERED SYNOVIAL VASCULAR EXPRESSION IN OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA) has historically been thought of as a degenerative joint disease, but inflammation and angiogenesis are increasingly being recognised as contributing to the pathogenesis, symptoms and progression of OA. ß-dystroglycan (ß-DG) is a pivotal element of the transmembrane adhesion molecule involved in cell-extracellular matrix adhesion and angiogenesis. Matrix metalloproteinases (MMPs) are the main enzymes responsible for cartilage extracellular matrix breakdown and are also implicated in both angiogenesis and ß-DG degradation in a number of malignancies. We aimed to investigate the expression and localisation of ß-DG and MMP-3, -9, and -13 within cartilage, synovium and synovial fluid and establish their roles in the pathogenesis of OA.

Methods: Following ethical committee approval, cartilage, synovium and synovial fluid were obtained from the hip joints of 5 osteoarthritic (patients undergoing total hip replacement) and 5 control hip joints (patients undergoing hemiarthroplasty for femoral neck fracture). The samples were analysed for ß-DG expression using Western Blotting and for the distribution of ß-DG, MMP-3, -9, and -13 using immunohistochemistry on paraffin embedded tissue.

Results: Whilst no significant expression of ß-DG was found in cartilage or synovial fluid, ß-DG was expressed in the smooth muscle of both normal and osteoarthritic synovial blood vessels. Moreover, ß-DG was expressed in endothelium of blood vessels of OA synovium, but not in the normal endothelium. In the endothelium of osteoarthritic synovial blood vessels, ß-DG co-localised with MMP -3 and -9. Degradation products of ß-DG were also identified within the synovium.

Discussion: Specific immunolocalisation of ß-DG within endothelium of inflamed OA blood vessels suggests that ß-DG may play a role in angiogenesis associated with OA. Its co-localisation with MMP-3 and -9, previously reported to also have pro-angiogenic roles, may be linked. Further research is required to understand these roles more fully.

P13

Abstract withdrawn

P14

EARLY NUTRITIONAL COMPROMISE AFFECTS THE MECHANICAL PROPERTIES OF SHEEP SPINES

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Introduction: Intrauterine protein restriction in rats is associated with low bone mass which persists with development through to adulthood. However, such adverse effects are not only restricted to bone. Intervertebral discs are the largest avascular structures in the body, and are particularly sensitive to their nutritional environment. We have examined the hypothesis that changes in the intervertebral disc (or ligaments), as a result of early nutritional compromise, affect the spine's mechanical properties.

Material and methods: Lumbar spines were removed from 8 sheep (6 male, 2 female: mean age 2.7 yrs) that had received different diets early in their development: two animals received a control diet, three received low protein in utero (IU), and three received low protein both in utero and postnatally (PN). Fifteen motion segments (consisting of two vertebrae and the intervening disc and ligaments) were dissected from the spines and tested on a hydraulically-controlled materials testing machine. Compressive stiffness and bending stiffness were measured before and after creep loading, in both flexion and extension. Reflective markers attached to the specimens were tracked during loading, enabling intervertebral angles to be calculated. Bending moment-angular rotation curves were used to calculate bending stiffness. Repeated measures ANOVA was used to test for differences in stiffness with posture and creep, and between the dietary groups.

Results: Compressive stiffness increased after creep loading ($p=0.002$) but was unaffected by posture or dietary group. In contrast, bending stiffness was unaffected by creep but differed significantly between groups and with posture. When compared to controls, bending stiffness in the IU group was reduced by 35% in flexion and 26% in extension ($p<0.02$). In the PN group, reductions of 28% in flexion and 15% in extension were observed ($p=0.056$).

Discussion: These results indicate that early protein restriction can affect the mechanical properties of the spine. These effects were evident in bending but not in compression, and tended to be greater in flexion than extension. These preliminary findings suggest that early protein restriction may affect the composition and mechanical function of the annulus fibrosus and the intervertebral ligaments which are the structures most involved in resisting flexion movements.

P15

THE USE OF VIBRATION-ASSISTED GRAFT COMPACTION IN IMPACTION BONE GRAFTING: IMPROVEMENT IN BONE GRAFT STRENGTH WITHOUT INCREASED RISK OF FRACTURE

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Background: Impaction bone-grafting in revision hip surgery generates high forces that may be transmitted through the graft to the femoral cortex, generating high surface strains and a concomitant risk of femoral fracture. Concern of inducing fracture may lead to under-compaction of the graft, with subsequent risk of implant migration. Vibration is commonly used in civil engineering applications to increase aggregate compressive and shear strengths. We have therefore examined the hypotheses that vibration-assisted graft compaction would (a) increase graft compaction compared with the standard femoral impaction grafting technique and subsequently reduce prosthesis migration and (b) reduce femoral hoop strains in the production of graft of a given density and mechanical properties.

Method: Physiological composite femurs were adapted to represent femurs encountered in revision hip surgery by widening of the internal diameter and thinning of the outer shell. In the control group, revision with the standard Exeter technique was simulated using highly washed morcellised bone graft from fresh-frozen human femoral heads. In the study group, vibration-assisted graft compaction was used. The femurs were mounted on a 5kN capacity load cell to measure the total force imparted during graft impaction. Strain gauges placed at the medial calcar and midshaft, measured hoop strains generated during the impaction process. On completion of graft impaction, an Exeter stem was cemented in place. Implant subsidence under physiological cyclic loading (5x 105 cycles) and graft density using micro CT were measured after compaction.

Results: There were no significant differences between the two groups in the peak forces (3.8-4.1kN) imparted during the impaction process. Similar peak hoop strains were observed in the both groups (1.2-1.4%). However a greater graft density was seen in the vibration group with minimal implant subsidence under cyclic loading.

Conclusion: The use of vibration during the impaction process allowed improved graft compaction to be achieved without increasing hoop strains in the femoral cortex. This has implications in preventing failure from under impaction without increasing the risk of fracture. Furthermore, this analysis is applicable to the study of novel synthetic grafts / mixtures in the impaction process for orthopaedic application.

P16

DEMONSTRATION OF CELLULAR CYTOTOXICITY IN UNWASHED FRESH FROZEN FEMORAL HEAD ALLOGRAFT AND COMPARISON TO WASHED GRAFT

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Fresh frozen femoral head (FFH) allograft is commonly used in impaction grafting for revision hip arthroplasty and long term success has been demonstrated by some groups. The optimum treatment of the graft prior to impaction has not yet been determined. Some groups wash the graft prior to impaction and others do not. Washing of the graft has been shown to improve bone ingrowth in a bone chamber animal model however the reasons for this remain unclear. The aim of this study was to identify any underlying cellular cytotoxicty of fresh frozen allograft bone before and after washing.

Samples of morcellised FFH allograft were taken during revision hip arthroplasties prior to impaction grafting. Paired samples, taken before and after washing were taken from each case. Washing was performed by 4 consecutive washes in 300ml warmed saline, the bone being filtered between each exchange of saline. Cytotoxicity was assessed for all samples using both contact and extract assays. Contact assays involved culture of cell lines in direct contact with bone samples. Extract assays utilised culture media conditioned with bone samples and subsequent quantitative assessment of cell metabolism and viability using both dimethylthiazol (MTT) and neutral red (NR) assays. All assays were performed using both human osteoblastic (MG63) and fibroblastic (HSF) cell lines.

Nine pairs of samples were analysed for cytotoxicity using both cell lines. Contact assays demonstrated a clear zone of cellular inhibition around the unwashed bone samples. Extract assays were performed in triplicate for each cell type and both MTT and NR assays giving 108 paired assay results. 88.9% of pairs (92/108) showed cytotoxicity in the unwashed sample. No washed samples demonstrated cytotoxicity. When grouped by assay and cell type, analysis of means showed statistically significant differences between washed and unwashed samples in MG63-NR ($p=0.0025$), HSF-NR ($p=0.0004$) and MG63-MTT ($p=0.008$). The difference observed in the HSF-MTT assays did not reach statistical significance ($p=0.06$).

In conclusion, we have shown that unwashed FFH allograft can be cytotoxic to human osteoblastic and fibroblastic cell lines in vitro. This suggests that allograft should be washed prior to impaction in order to optimise the biological compatibility.

P17

COVALENT BONDING OF LAMININ-5 TO TITANIUM ALLOY: A RADIOISOTOPE STUDY

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Introduction: Intraosseous Transcutaneous Amputation Prostheses (ITAP) could overcome the problems associated with conventional stump-socket prostheses for amputees (pressure sores, pain, infections and unnatural gait), by attaching the external prosthesis directly to the skeleton via a skin penetrating abutment. Despite this, the skin breach introduces a potential route for infection. For success, a biological seal at the skin-ITAP interface is essential.

The protein Laminin-5 (L-5) is a 'biological glue', which is integral to epitheial cell adhesion. Covalently bonding L-5 to the ITAP titanium alloy (Ti6Al4V), may enhance the strength of the skin-ITAP interface.

Silanisation, a chemical technique that covalently bonds proteins to metals, could be used to bond L-5 to Ti6Al4V. We have assessed the characteristics L-5 silanised Ti6Al4V as a potential substrate for ITAP.

Method: To determine the maximum quantity of L-5 that could be silanised to Ti6Al4V, and its relative stability when soaked in foetal calf serum (FCS) over time; polished Ti6Al4V discs were silanised by immersing in aminopropyltriethoxysilane followed by glutaraldehyde. Radiolabelled rat laminin-5-I125 was then added. Discs were immersed in FCS for 4 days (37 C) and analysed at 24 hour intervals in a liquid scintillation counter. Un-silanised discs were used as controls.

Results: L-5 was successfully covalently bound to Ti6Al4V. 10ng, 100ng, 250ng and 500ng droplets yielded significantly more silanised L-5 ($p<0.05$), but no difference was observed between 750ng and 1000ng. Percentage L-5 covalently bound ranged from 33% and 65%.

A small decrease in bound L-5 occurred after 24 hours of FCS soaking ($p<0.05$), but subsequent to this no significant reduction was observed for 4 days ($p<0.05$). Controls showed a significantly larger reduction after 24 hours ($p<0.05$).

Conclusion: Covalently bonding L-5 to Ti6Al4V by silanisation can be achieved with predictable results. Large enough quantities can be immobilised to influence cellular function. L-5 silanised to Ti6Al4V remains stable in vitro over time and is not removed. Following the study of cellular interactions with silanised L-5, a stable skin seal may be achieved at the transcutaneous portion of the ITAP.

P18

A BIOMECHANICAL INVESTIGATION OF PROBLEMS ASSOCIATED WITH NEWER INTRAMEDULLARY NAIL DESIGNS

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Introduction: For years traditional intramedullary nails (IMNs) have been used with great success to treat long bone fractures, however, based upon our clinical observations, we hypothesise that design changes incorporated into newer femoral IMNs reduces fracture stability resulting is a higher incidence of non-union.

AIMS

To biomechanically test the factors that may reduce fracture stability.

Materials and methods: The fracture fixation model consisted of custom made stainless steel IMNs of different wall thicknesses and outer diameters, cylinders manufactured from stainless steel, aluminium or HDPE of differing inner diameters and wall thicknesses, and 5mm rods made from stainless steel or titanium. The dimensions of the cylinders were chosen to resemble those commonly observed in the distal femur. The test nails and cylinders were connected using a single rod. Axial loading was undertaken up to 2KN (constant rate of 0.5KN/sec) and repeated a minimum of three times. The effects of various factors such as IM nail wall thickness and outer diameter, the alloy from which the rods were manufactured, and, the diameter, wall thickness and material properties of the cylinders were studied.

Results: The factors that most affected stability were the diameter, wall thickness and the material properties of the cylinders, with the least stable configuration being a HDPE cylinder with a diameter of 75mm and a wall thickness of 3mm. By reducing the diameter of the cylinder to 50mm combined with increasing the wall thickness to 5mm, stability increased considerably even when HDPE was used. The stability of each fracture fixation system was further reduced by using titanium rods.

Discussion: In clinical practice, new femoral IMNs permit longer cross screws to be inserted in the distal femur where the diameter is greatest and the cortical bone is thinnest. Since cancellous bone offers little resistance, screws effectively span from one cortex to the other gaining limited purchase in the bone. As a result, the newer IMN systems are more likely to displace regardless of the direction and force applied. This effect is exaggerated by using titanium. Overall the combination of screw length, choice of alloy and cortical thickness could easily explain our unsatisfactory clinical observations.

P19

A 3-DIMENSIONAL, IN VITRO MODEL TO STUDY THE EFFECTS OF COMPRESSIVE LOADING ON OSTEOBLASTIC CELLS

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Mechanical force is an osteoinductive factor that plays an important role in bone growth and repair in vivo (Carter et al. 1988). Many in vitro studies have shown that osteoblasts and osteocytes respond to mechanical loads such as stretch and fluid-flow induced shear stresses, with initiation of signalling pathways (Reilly et al 2003). The underlying mechanisms by which bone cells respond to mechanical signals are difficult to investigate in a 3-D environment, because of reduced nutrient delivery to cells and difficulties in analysis.

We are developing a model to analyse the effects of mechanical compression on matrix forming osteoblasts in a 3-D system. Our model uses polyurethane (PU) open cell foam scaffolds, MLO-A5 osteoblast-like cells (Kato et al 2001) and a sterile fluid filled biodynamic loading chamber (Bose). We have shown using a cell proliferation assay (Promega) that cells survive well and proliferate in the PU scaffolds. Cell number after 15 days of culture was four times that after 5 days of culture. To examine the effect of mechanical stimulation on osteoblastic cells we seeded MLO-A5, kindly donated by Dr. L. Bonewald, at densities of 125,000 cells per scaffold in PU foam cylinders, 10 mm thick and 25 mm diameter. The cell seeded PU scaffolds were dynamically loaded in compression at 1Hz, 5% strain in a sterile fluid-filled chamber. Loading was applied for 2 hours per day at days 5, 7 and 9 of culture. In between loading cycles, scaffolds were cultured in an incubator in standard conditions.

Preliminary data indicates that the cells survived loading but final cell number was reduced compared to unloaded controls by 30%. However, the scaffold stiffness (Young's modulus) increased in loaded samples over time (days) which may be an indication of increased matrix production. Fluorescence microscopy indicated that loaded cells were distributed in dense clusters whereas unloaded cells were distributed evenly throughout the scaffold. In conclusion, this model has the potential to answer questions about cell survival, distribution and matrix production in 3-D, in response to mechanical signals.

P20

COMPARISON OF PRESSURES GENERATED BY VARIOUS INTRAMEDULLARY REAMERS IN USE IN THE NHS TODAY

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Reamed, locked intramedullary nailing is the treatment of choice for many long bone fractures, be them open or closed injuries. Certain nails used can be inserted without any locking component or reaming. However, the most biomechanically sound fixation is achieved with a reamed, locked nail, and this therefore is the preferred construct. The process of reaming is not without complication, however. Pulmonary complications secondary to embolization of intramedullary contents are of the most concern. The formation of emboli is believed to be a direct result of raised intramedullary pressures created largely by the reaming process, although nail insertion does also play a part.

The magnitude of intramedullary pressures generated during the reaming process is due, in part, to the design of the reamer itself. This study compares four different reamers currently in use in NHS hospitals today. The reamers used include an older design (AO Universal(TM)) and three newer designs (Synthes Synream(TM), Biomet 5+(TM) and Stryker Bixcut(TM)).

Four different reamer head sizes were used- 9.5mm, 11.5mm, 13.5mm and 15.0mm. These were tested in vitro using a Vaseline(TM)/paraffin oil mixture to simulate intramedullary tissue and Perspex(TM) tubing of varying sizes to simulate a long bone with an intramedullary cavity.

The results showed that the older generation reamer produced consistently higher pressures than the newer designs of reamer with statistical significance. All the newer generation reamers produced similar pressure magnitudes, although the Biomet 5+(TM) tended to produce the lowest pressures with some statistically significant differences.

This study shows that different designs of reamer can generate different pressures and that the newer generation of reamers do produce lower pressures. This is therefore important in the prevention of complications associated with reaming and intramedullary nailing.

P21

PATELLAR TENDON OR PATELLAR LIGAMENT? A COMPARATIVE STUDY IN AN OVINE MODEL

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Tendons and ligaments are similar in composition but differ in function. Simple anatomical definitions do not reflect the fact individual tendons and ligaments have unique properties due to their adaptation to a specific role. The patellar tendon is a structure of particular clinical interest. A null hypothesis was declared stating that the patellar tendon is not significantly different in terms of matrix composition and collagen fibril diameter to other tendons.

The lateral and medial collateral ligaments (LCL, MCL), anterior and posterior cruciate ligaments (ACL, PCL), together with the long digital extensor, superficial digital extensor and patellar tendons (LDET, SDFT, PT) were harvested from 3 cadaveric ovine hindlimbs. The extracellular matrix was assessed in terms of water, collagen and total sulphated glycosaminoglycan (GAG) content. The organisation of the collagen component was determined by an ultrastructural analysis of collagen fibril diameter distributions using electron microscopy, together with values for the collagen fibril index (CFI) and mass-average diameter (MAD).

There were significant differences between ligaments and tendons. The PT had a bimodal collagen fibril diameter distribution with CFI 72.9%, MAD 202nm, water content 53.1%, GAG content 2.3 g/mg and collagen content 73.7%, which was not significantly different from the other tendons.

The results of this study support the null hypothesis suggesting that the patellar tendon is similar to other tendons and demonstrate that tendons have different characteristics to ligaments.

P22

A COMPARISON OF THE IN-VITRO BIOMECHANICAL PERFORMANCE OF A COMPOSITE BIO-ABSORBABLE SCREW AND CONVENTIONAL METAL SCREWS FOR SCAPHOID FRACTURE FIXATION

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Objective: To compare the ability of a new composite bio-absorbable screw and two conventional metal screws to maintain fixation of scaphoid waist-fractures under dynamic loading conditions. Methods: Fifteen porcine radial carpi, whose morphology is comparable to that of human scaphoids, were osteotomized at the waist. Specimens were randomized in three groups: those in group I were fixed with a headed metal screw, in group II with a headless tapered metal screw, and in group III with a bio-absorbable composite screw. Each specimen was oriented at forty-five degrees and cyclically loaded using four blocks of 1000 cycles, with peak loads of 40, 60, 80 and 100 N, respectively. In case of gross failure the number of cycles to failure was determined. Otherwise, permanent displacement at the fracture site was measured after each loading block from a standardized high-magnification photograph using image analysis software (Roman v1.70, Institute of Orthopaedics, Oswestry). Statistical analysis was by ANOVA and tolerance limits.

Results: No gross failure occurred. Average displacements after 4000 cycles up to 100N were 0.05mm±0.03SD (headed metal), 0.15mm±0.16SD (headless metal) and 0.29mm±0.11SD (composite) and differed significantly ($p<0.02$). Using tolerance limits, the data allowed us to predict that with 95% certainty, displacement in 95% of any sample fixed with a headed metal screw will be below 0.17mm, headless metal screw below 0.84mm, and composite screw below 0.76mm.

Conclusion: Comparing two types of conventional metal screws and a new composite bio-absorbable screw to maintain scaphoid fixation under cyclic loading conditions, we found small average fracture displacements for all three screws. Moreover, even following severe cyclic loading conditions, clinically meaningful displacements of more than 1 mm are highly unlikely for any of the three screws. We therefore conclude that a new bio-absorbable composite screw can serve as an alternative to conventional screws when fixing scaphoid fractures.

P23

COLLAGEN GEL COMPRESSION DURING INITIAL CULTIVATION ENHANCES CELL DISTRIBUTION AND SCAFFOLD STABILITY OF OSTEOCHONDRAL CONSTRUCTS

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Introduction: Homogenous cell distribution and sufficient initial scaffold stability remain key issues for successful tissue engineered osteochondral constructs. The purpose of this study was to investigate the application of initial compression forces during the first 24 hours of cell culture followed by different stress patterns.

Methods: Bone marrow stromal cells were harvested from the iliac crest during routine trauma surgery. The cells were expanded in a 2-dimensional culture and then seeded into the biologic hybrid scaffold with a concentration of 1x10E6 cells per ml. Pressure and vacuum forces were applied in a specially developed glass kit. The constructs were exposed to two different protocols of compression combined as oteochondral matrices of CaReS (collagen I) and Tutobone (Ars Arthro, Esslingen, Germany and Tutogen Medical GmbH, Neunkirchen a. Br., Germany). Controls were resected osteochondral fragments from patients with articular fractures and uncompressed constructs. These effects were evaluated using light microscopy after standard staining to identify matrix penetration. Biomechanical tests were conducted, too using a modified biomechanical testing machine. The 'constrained compression', maximum load to failure, modulus, and strain energy density were determined.

Results: Histology: Penetration and cell distribution was demonstrated homogenous and vital, respectively. Mechanical tests showed a significant enhancement of primary matrix stability. The following stress patterns did not enhance significantly stability over seven days.

Discussion: The aim of this project was to investigate the response and cell distrubution of human bone marrow stromal cells seeded on a 3-dimensional biologic hybrid scaffold using compression and vacuum forces.

The integration of mechanical stimulation in the tissue engineering process may lead to a progress in the structural and biomechanical properties of these tissues and offers new possibilities in the management of bone injuries and degenerative diseases.

P24

A CADAVERIC STUDY TO EVALUATE THE ROLE OF THE SCAPHOLUNATE INTEROSSEOUS LIGAMENT AND THE RADIOSCAPHOLUNATE LIGAMENT IN THE SCAPHOLUNATE KINEMATICS

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The pattern of injury to the carpal ligaments following wrist trauma is unclear. Different imaging techniques often prove inconclusive rendering the diagnosis difficult and hence the treatment controversial. This study aimed to observe and evaluate the differences in scapholunate kinematics before and after sectioning the scapholunate interosseous ligament (SLIL) and radioscaphocapitate ligament (RSC).

Twenty two embalmed cadaveric wrists were used. There were four males and seven females with an average age of 84 years. Their medical records confirmed the absence of previous history of wrist diseases or injuries. The extensor and flexors tendons of the wrist were removed leaving the capsule intact. Two drill bits (1.5 mm) were used to make a hole each in scaphoid and lunate, one centimeter apart. The drill bits were left in the bones to act as metal wires for calibration. Each wrist was moved through a set of motions and each movement was performed thrice; first one with the ligaments intact, second with SLIL sectioned and the last one with RSC excised. Digital photographs were taken and angles measured with MB Ruler software. Analysis of variance was done using SPSS 12.

There was no angle between the metal pointers when the ligaments were intact. There was movement and change in angle detected when SLIL and RSC were sectioned. The sectioning of the SLIL lead to a significant increase in the angle between the pointers in all the movements recorded (p value <0.001). Subsequent sectioning of the RSC further increased this angle but this increase was much smaller compared to that after sectioning SLIL. On completion of the measurements the wrist capsule was opened to reveal that both the ligaments had been successfully sectioned and there were no degenerative changes in the bones or ligaments in any wrist.

This first cadaveric evaluation of alterations in scapholunate motion with sectioning of SLIL and RSC revealed that SLIL has a significant influence on the scapholunate kinematics, where as sectioning of the RSC has little additional effect. This in-vivo finding might have implications of importance of preserving SLIL during wrist surgeries and its role in management of carpal instabilities.

P25

SIMULATION OF FRACTURE IN THE CEMENT MANTLE AND AT THE BONE-CEMENT INTERFACE IN RECONSTRUCTED ACETABULA

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Multiple biological and mechanical factors may be responsible for the failure of fixation in cemented total hip replacements (THRs). Although the eventual failure of THRs may appear to be biological, the initiation of the failure during early period post operation may well be mechanical. It is in this area that mechanistic analysis is of particular significance.

This study builds on work by Rapperport et al, Dalstra and Huiskes on stress analysis of implanted acetabulum, while focuses on fracture mechanics analyses of fracture of cement and of bone-cement interface. Specifically, finite element models were developed where cracks of most favourable orientations in the cement mantle were simulated. Possible crack path selections were explored. A simplified multilayer experimental model was also developed to represent the implanted acetabulum, and fatigue tests were carried out on the model. The experimental results were compared with those from the FE model.

Furthermore, interfacial crack growth at bone-cement interface was simulated from the superior edge of the acetabulum, as suggested from the clinical observations. The strain energy release rates were computed for typical hip contact forces during gait and as a function of crack length. Associated phase angles were also computed to account for the materials mismatch. The results were evaluated against the interfacial fracture toughness of the bone-cement interface, measured using sandwich Brazilian disk specimens. The results show that although interfacial fracture seems to be unlikely for large phase angles where shear component is most active, the strain energy release rates are comparable with the values of the interfacial fracture toughness when mode I is predominant, suggesting interfacial fracture.

The study also shows that the fracture toughness of cement is much higher than the interfacial fracture toughness of bone-cement, this may explain the reason why interfacial fracture is favoured even if the crack driving force at bone-cement interface appears to be weaker than that in the cement mantle.

P26

INTRODUCING GEOMETRIC MORPHOMETRICS TO COMPARE ANATOMICAL VARIATION IN BONE SHAPE AND THE POTENTIAL APPLICATION 3-DIMENTIONAL RECONSTRUCTIONS OF BONES FOR FURTHER STUDIES

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The aim of this study was to discover if the ulnar styloid is sufficiently consistent in size, shape and position relative to other bony features of the ulna to be used as a reference in pre-operative planning of fixation of broken bones.

The comparison of size and shape (together known as form) between bones has recently been facilitated thanks to the advance of technologies designed to allow the comparison of the form of structures using anatomical landmarks.

This new class of methods is collectively known as geometric morphometrics. It eliminates the differences in location and rotation of landmark through registration that minimises the sum of squared deviations from each other after scaling. This is Procrustes registration. The residual size and shape information is amenable to statistical analysis. In the present application, the registered

Procrustes landmarks are used to compute a mean (reference) shape. The individuals are then compared to this mean/ reference shape. Using principal components analysis (PCA) variations in shape are not only identified, but also quantified. The identification of patterns of deviation from the mean shape is considerably enhanced through the use of 3-D visualistaions of the shape variations represented by the space of the PCA.

These analyses indicate that the ulnar styloid is sufficiently consistent in location to other anatomical landmarks that it could be used as a radiographic marker in preoperative planning.

More importantly, the analysis of this study indicates that the methods of geometric morphometrics are widely applicable to the analysis of 3-D variations in morphology facilitating the analysis and comparison of radiographs. A useful future application will be in the development of 3-D reference morphologies that will allow the surgeon to compare and contrast the morphology of a radiograph of a badly broken (comminuted) bone to a standard one. Eventually computer might assist the surgeon by geometrically and visually showing how and by how much the bone needs reduction. Similarly, applications to the the virtual comparison of diseased and healthy bones might allow quantative and visual comparisons that could aid diagnosis and planning.

P27

A COMPARISON OF BONE FRAGMENT DISPLACEMENT IN TWO DISTAL RADIUS ORIF TECHNIQUES

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Two stainless steel 'TriMed' distal radial fracture reduction techniques were tested to compare the relative stability of the two in vitro for a pre-determined fracture pattern. The movement of the bony segments were plotted over time using an ARAMIS 3 dimensional non-contacting displacement mapping system (GOM mbH, Braunschweig, Germany) to give quantitative data. The data was used to calculate the relative motion of the bony segments with the aim of investigating regions of compression across the fracture line, which is thought to accelerate fracture healing, and shear between bony segments, which is detrimental to fracture healing.

Ten third generation adult radius biomechanical model Sawbones (Sawbones, Malmö, Sweden) were cut to simulate AO type C2 fractures with dorsal comminution. Five bones were plated using the TriMed fixed angle volar bearing plate and five were plated using the TriMed radius and ulnar plating technique. Samples were potted and loaded cyclically at 1 Hz via a floating scaphoid-lunate bearing onto the end of the radius at incrementally increasing loads of 100 N - 500 N with 1000 load cycles applied for each load level.

The results showed the radius and ulnar pin-plate configuration allowed greater movement of the articular surface, with relative shear motion and separation between the two segments, although the relative shear movement between the two distal segments was below 2mm, which is considered the definition of failed fixation. With the volar bearing plate the two distal segments moved as single unit and compression with minimal shear was applied across the fracture line to the proximal radius. Thus the radius and ulnar plates allowed shear across all three fracture lines, while the volar plate held the two distal segments fixed relative to each other and allowed compression across the interface with the proximal radius. The ARAMIS system allowed the three dimensional motion of the bony segments to be followed, in particular the relative motion between the segments, indicating the type of healing to be expected clinically. The study demonstrated the value of ARAMIS in investigating the stability of wrist fractures fixations and can easily be adapted to investigate other orthopaedic fixation systems.

P28

GEOMETRY OF THE PROXIMAL ARTICULAR SURFACE OF THE HUMERUS AS IT RELATES TO THE GLENOID

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Introduction: Successful shoulder arthroplasty is based on restoration of the individual's proximal humeral morphology with a precise osteotomy of the humeral head at the level of the anatomical neck. The objective of this study was to determine the geometry of the articular portion of the humeral head in contact with the glenoid in the neutral position and compare the orientation to the geometry of the humeral head determined using the cartilage/calcar interface of the anatomical neck.

Methods: An intact rotator cuff and joint capsule were exposed for six cadaveric full arms. Precision perspex reference cubes were attached to the greater tuberosity of the humerus and to the coracoid process of the scapula on each specimen. Each shoulder was mounted in a custom built jig with the arm fixed in the neutral position and a Microscribe 3D-X digitizer used to digitize three faces of each precision cube. The shoulder joint was then disarticulated and both the humerus and scapula re-mounted on the same jig, independently. The cube faces were re-digitized and relevant points, lines and surfaces were identified and digitized on each humerus and scapula. The humeri were then scanned using a high precision surface laser scanner.

The data collected from both digitizing tools were merged into the same coordinate system and graphically represented. Paired Student's t-tests were used to compare the inclination and retroversion angles for the two techniques.

Results and discussion: The study found a significant difference in inclination (p less than 0.02) and no difference in retroversion (p equal to 0.75) when the glenoid position was used to calculate humeral head orientation (Inclination: Mean 11.5 deg., StD. 11.2 deg.; Retroversion: Mean 20.5 deg., StD. 6.6 deg.) when compared to using the cartilage/calcar interface (Inclination: Mean 134.1 deg., StD. 1.9 deg.; Retroversion Mean 21.7 deg., StD. 13.9 deg.).

Small deviations in the recovery of head orientation in shoulder arthroplasty may impact on the longevity of an implant. The differences in inclination and retroversion noted in this study may alter the load on the glenoid and/or rotator cuff mechanism in joint replacement. Further research is necessary.

P29

COMPRESSIVE LOAD-BEARING BY THE APOPHYSEAL JOINTS AND UNCOVERTEBRAL JOINTS IN THE CERVICAL SPINE

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Introduction: Vertebral bodies and intervertebral discs resist most of the compressive force acting on the spine. However, experiments on lumbar spines have shown that apophyseal joints can resist more than 50% of applied compression, and that the proportion varies with spinal level, disc narrowing, and posture. In the cervical spine, the situation is likely to be complicated by the presence of uncovertebral joints on the lateral margins of the disc. Load-sharing is important because it influences injury risk, and predisposition to degenerative changes. The present study aims to characterise compressive load-sharing in the cervical spine.

Methods: Sixteen cervical motion segments, consisting of two vertebrae and the intervening disc and ligaments, were dissected from nine cadaveric spines, aged 48-77 yrs (mean 63 yrs) which had been stored at -17degC. Specimens were subjected to 200N of compression while the distribution of compressive 'stress' was measured along the mid-sagittal diameter of the disc, using a pressure transducer side-mounted in a 0.9mm-diameter needle. 'Stress profiles' effectively were integrated over area to calculate the total compressive force acting on the disc. Experiments were performed with each specimen in flexion, extension and neutral posture. They were repeated after creep compressive loading (2 hrs at 150N) to simulate diurnal loading in life, and again following removal of the apophyseal joints. Eight specimens were re-tested following bi-lateral removal of the uncovertebral joints.

Results: Creep loading reduced disc height by an average 0.64mm (approximately 12%). Creep reduced overall computed disc loading by 14% and 25% in neutral and extended postures respectively ($P<0.005$). Apophyseal joint removal increased disc loading in extension (only) by 14% ($P<0.05$). Uncovertebral joint removal further increased disc loading in flexed, neutral and extended postures by 28%, 33% and 21% respectively ($P<0.05$).

Conclusion: Creep loading of the cervical spine transfers loading to the apophyseal joints and uncus. The former effect is small, and significant only in extended postures. The latter effect is larger, and is greatest in flexed and neutral postures.

P30

IMPROVED KNEE KINEMATICS IN POSTTRAUMATIC NON-ANATOMIC ARTICULAR GEOMETRY: APPLICATION OF COMPUTER ASSISTED ORTHOPEDIC SURGERY USING ORTHOPILOT SOFTWARE

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Malpositioning of the component of a total knee implant and malalignment of the leg is one of the significant factors for the outcome after Total Knee Arthroplasty.

Previous studies have shown that the use of a navigation system can improve these. This article presents the initial results of a prospective and non-randomised study describing navigated implantation in TKA with special reference to soft tissue balancing in knees with posttraumatic deformity. The secondary objective is to found out reproducibility of the software.

Methods: Since January 2004, 15 patients with posttraumatic arthrosis of the knee and axial malalignment of more than 15 degrees, pre operative arc of motion 75 degrees admitted to our senior author for TKA have been followed up prospectively. The data were collected over a period of 25 months. Apart from the usual clinical evaluations, no patients had CT of the leg prior to the operation & postoperatively. Intra-operative and peri-operative morbidity data were collected and blood loss measured.

Results: A postoperative leg axis between 3 degrees varus and 3 degrees valgus was obtained in all of the navigated knees after soft tissue balancing. The alignment of the components using computer-assisted surgery in regard to femoral varus/valgus, femoral rotation, tibial varus/valgus, tibial posterior slope, tibial rotation are reproducible and consistent. Computer-assisted surgery took longer with a mean increase of 31 minutes for kinematic data acquition. Intraoperatively we achieved range of motion more than 120 degrees. No patient required manipulation postoperatively for improving range of motion

Conclusion: These results support that the precise surgical reconstruction of the mechanical axis of the knee and proper alignment of the component is achievable in patients who suffered posttraumatic deformities and secondary arthrosis by using an intraoperative navigation system.

It has been mentioned in the literature that minor deviations in the insertion point of Intramedullary instrumentation during TKA may result in malalignment of several degrees [Nuno-Siebrecht 2000], which can be avoided with these soft ware.

P31

BONE CEMENT CREEP AND POROSITY: THE EFFECT OF ANTIBIOTICS?

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The visco-elastic behaviour of acrylic bone cement is a key feature of cement-implant performance. The ability of the cement to creep in conjunction with a force-closed design of stem (collarless polished taper) affords protection of the vital bone-cement interface. Most surgeons in the UK use antibiotic-laden PMMA in primary total joint arthroplasty. In revision surgery the use of bespoke antibiotic-cement combinations is common.

The aim of this study was to elicit the effect of antibiotics upon the physical properties of bone cement.

Methods: The static properties of the cements were assessed following protocols described in ISO 5833: 2002, while the viscoelastic properties of the cement were measured with in-house developed apparatus in quasi-static conditions. Creep tests were performed in four point bending configuration over a 72 hour period in physiological conditions. Porosity was measured on the mid cross section of the creep samples using a digital image technique.

The cements used were Palacos R40 and Palacos R with gentamicin. The antibiotics added included fucidin, erythromycin, teicoplanin and vancomycin in 500mg powder aliquots up to a maximum of 1g per 40 g mix.

All data were analysed using ANOVA with Bonferroni post-hoc test. Pearson's correlation coefficient was used to investigate the association between physical factors (SPSS).

Results: The static and working properties did not vary significantly with antibiotic additions. The mean creep of the cement increased in line with the amount of antibiotic added. The specific antibiotic was not relevant. The differences were statistically significant. Mean porosity also increased with antibiotic mass. There was a linear relationship between cement porosity and creep!

Conclusions: Despite modern mixing techniques the porosity of bone cement increases with antibiotic additions. This increased porosity is related to the greater creep seen in the cement. Surgeons should apply these findings when planning revision hip surgery.

P32

THE BENEFICIAL EFFECT OF MUSCLE ACTION ON STRESS DISTRIBUTION IN THE PELVIS

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Previous experimental studies of the pelvis have been carried out on cadaveric samples stripped of soft tissue. Investigations of the stress concentrations present in the pelvis due to the application of force through the hip joint have been conducted with the superior iliac crests cast in resin or cement. Thus stress concentrations are observed towards the superior iliac crests, and to some extent the pubic symphysis (these being the areas in which force transfer can occur). Due to the rigid fixing of the pelvis in these experiments, the pelvic bone has become viewed as a 'sandwich beam' acting between the sacro-iliac and the pubic joints. Numerical models employing similar fixed conditions have shown good agreement with the experimental studies.

However it is clear that these experiments, and the accompanying computational models are not representative of the in-vivo situation, in which the muscles and ligaments of the pelvis and hip joint provide resistance to movement, and in the case of muscles place additional forces on the pelvis, not addressed in the experimental studies. This study presents a finite element model of the pelvis in which novel techniques have been used to include the pelvic ligaments, and hip joint muscles using realistic attachment areas on the cortex, providing a more realistic comparison to the in-vivo environment. Joint interactions at the pubic symphysis and sacro-iliac joints are also simulated. A fixed boundary condition model is also presented for comparison.

The resulting stress concentrations in the pelvis for single leg stance observed in the in-vivo boundary condition model are dramatically different to those presented in studies in which the pelvis is rigidly fixed in place. The abductor muscles are seen to play a significant role in reducing stress concentrations towards the sacro-iliac joints and superior to the acetabulum, in comparison to fixed boundary condition analyses. Stress reductions away from the acetabulum are also observed in the underlying trabecular bone for the in-vivo boundary condition model. Similar stresses are observed within the acetabular region for the fixed, and in-vivo boundary condition models.

P33

DETERMINING IN-VIVO PERFORMANCE OF METAL ON METAL BEARING HIP ARTHROPLASTY WITH EXERCISE RELATED RISE IN COBALT LEVELS

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In-vitro studies have shown that wear rates of the metal on metal (MOM) bearing hip prostheses decline once the bearing runs-in and the bearing subsequently enters a steady state wear phase. Baseline cobalt levels are thus expected to decline with time in patients. Several clinical studies have not found such a decline. Baseline cobalt levels are hence limited in their capacity to provide information on the wear performance of the bearing couple. We have demonstrated in a previous study that exercise causes a rise in plasma metal ion levels in patients with MOM bearing hip replacement. Would the exercise related cobalt rise be more sensitive to detect change in wear behaviour of the bearing couple? We tested the alternate hypothesis that exercise related rise in the plasma cobalt levels will correlate inversely with the duration of MOM implantation.

Sixteen patients with three different well functioning MOM bearing hip replacement [two types of resurfacing (BHR, Cormet) and

Metasul] were included into the study. Patients were divided in to two groups based on time since implantation, an early group of mean 18 months and a late group of mean 57 months. Plasma levels of cobalt were measured before (baseline) and after 1 hour of maximal exercise (peak). The difference between baseline and peak for each patient provided the exercise related cobalt rise. A significant increase in plasma cobalt levels of 13% was noticed after the exercise ($p < 0.005$). Baseline Cobalt levels in the late group (53nmol/l) were higher than early group (44nmol/l) but the difference was not significant ($p=0.45$). However, the mean exercise related Cobalt rise levels was lower in late group (3.5nmol/l) than the early group (6.5nmol/l). This lower rise in cobalt level in the late group precisely reflects on the steady state wear as seen in in-vitro tests.

Baseline cobalt levels are limited in determining the in-vivo performance of the bearing couple. Exercise related rise in cobalt levels can differentiate the running in and steady state wear phases of metal on metal bearings and is thus a more accurate tool of assessing in-vivo wear performance of the bearing couple.

P34

CUP INCLINATION ANGLE IS POSITIVELY CORRELATED WITH WHOLE BLOOD CONCENTRATIONS OF COBALT AND CHROMIUM IONS AFTER METAL-ON-METAL HIP RESURFACING

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Background: Metal-on-metal bearing hip replacements release between three and nine times more cobalt and chromium ions than a metal on polyethylene bearing hip replacement. We do not fully understand the cause for the variability of ion levels after metal on metal hip replacement. The factors that determine an individual's levels of metal ions include: firstly, patient factors (renal failure, patient weight, high activity); secondly, manufacture factors (head size (and fluid film lubrication), carbide density, surface finish) and lastly study factors (bilateral implants, time from operation). Biomechanical studies suggest that component position, in particular acetabular inclination, is important for wear rate but there is no published correlation from clinical studies.

Aim: To investigate the relationship between acetabular inclination angle and metal ion levels of patients with Birmingham Hip resurfacings.

Methods: Using standardised radiographs, we measured the inclination angle (using UTHSCSA image tool) of the acetabular components in thirty-one patients (mean age 54 years) who underwent unilateral Birmingham hip resurfacing (mean time post operation of 22 months). We also measured peripheral whole blood chromium and cobalt ion concentrations using inductively coupled mass spectrometry. All components were well fixed.

Results: There was a positive correlation between the inclination angle (range 28 degrees - 55 degrees) of the acetabular component and whole blood concentration of Cobalt (range 2.3 - 7 mcg/L), Chromium (range 0.56 - 4.3 mcg/L) and total metal ion levels (range 3.1 - 10.3 mcg/L). This finding was statistically significant, with a Pearson correlation coefficient of 0.46 (95% CI 0.13-0.70) and a p-value of 0.00398.

Conclusion: Acetabular inclination angle is likely to be a factor in determining an individual's metal ion levels in patients with metal on metal resurfacing. We also identified a threshold level of 50 degrees inclination, after which the metal ion levels rise dramatically. We describe the possible biomechanical mechanisms to explain these results. We recommend surgeons implant the metal socket at an inclination angle of less than 50 degrees.

P35

THE EFFECT OF THE CEMENT THICKNESS ON THE ACETABULAR STRAIN DISTRIBUTION NEAR THE BONE-CEMENT INTERFACE

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Objective: To examine the effect of varying the thickness of the cement mantle on the strain distribution near the bone-cement interface.

Background: An insufficient cement mantle is thought to generate cement fractures near the bone-cement interface. Debonding at the bone-cement interface may accompany such fractures, and, mechanical failure of the prosthesis may follow. In this study, we aim to analyse the relationship between the cement mantle thickness and the acetabular strain distribution near the bone-cement interface.

Experimental model: Four hemi-pelvic saw bones specimens were implanted with six protected precision strain gauges. All specimens were prepared to receive a 53/28 cemented polyethylene cup (Depuy Charnley Elite).

Methods: We simulated hip joint force relative to the cup during normal walking for quasi-static tests on an Instron 1603 testing machine. The magnitude of the maximum and minimum principal strains, and the orientation of the maximum principal strains were calculated based on the readings of strains from a 32 channel digital acquisition system.

Results: Statistically significant differences in the total strains per gait cycle ($p<0.001$) have been noted at all gauge locations. In the principal load bearing quadrants, the recorded tensile strains are reduced by 50% as a result of the thicker mantle, while the transmission of compressive strain is enhanced.

Conclusion: A cement mantle thickness of 5-6mm may preserve the structural integrity of the principal load bearing quadrants of the acetabulum better than a mantle thickness of 2-3mm, by minimising the acetabular strains. This maybe desirable in total hip replacements for conditions such as rheumatoid arthritis and osteoporosis, where the poorer quality bone can be assisted by recruitment of a larger surface area to participate in load bearing.

Keywords: Principal strains; Cement mantle; Mantle thickness; Bone-cement interface; Acetabular strains.

P36

PROXIMAL FEMORAL PRESSURISATION DURING STEM INSERTION OF A CEMENTED HIP REPLACEMENT: AN IN-VITRO COMPARISION OF THREE MODALITIES

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Background: Inadequate proximal femoral pressures obtained during a cemented, primary hip replacement may lead to poor stem fixation. Proximal occlusion during stem insertion,may help in achieving a uniform and sustained rise in intra-medullary pressures, distally and proximally. High intra-medullary pressures correlate with better cement penetration and increased cement-bone interface push-out strength.

Methodology: An In-vitro analysis of femoral pressures was performed. A femoral medullary cavity was created in plaster of Paris constrained in an aluminium cylinder. Intramedullary pressures were measured via pressure transducers.High viscosity bone cement (Palacos-R) was gunned into the medullary cavity. No.3 Exeter stem was inserted with no proximal occlusion, with thumb occlusion over the calcar and with the Exeter Horse-collar. Experiments were repeated by delaying the timing of insertion and with lower viscosity cement (Simplex-P). A small series of experiments were done to ensure that that the stem insertion was performed at standard cement viscosity. The experiments were carried out with the same viscosity of Palacos-R at 4 minutes and Simplex-P at 6 minutes. Palacos-R at 4 minutes 30 seconds had a higher viscosity.

Results: A total of 54 experiments were performed. Of these 18 experiments were done with Palacos R cement, with the stem inserted early on in the curing phase and 18 with a delayed time of insertion. The last 18 experiments were performed with Simplex P cement with the stem inserted early on in its curing phase.

Intramedullary pressures were better in all zones, for all cement modes, with proximal occlusion. The highest pressures were seen with Palacos-R at 4 minutes 30 seconds with proximal thumb occlusion. Stem insertion into Palacos-R at 4 minutes or 4 minutes 30 seconds, gave higher pressures than Simplex-P, with or without any form of occlusion. With Simplex-P, intramedullary pressures were higher, with Collar rather than thumb occlusion.

Conclusion: Occluding the medial cal car area during stem insertion, is an effective way of achieving and sustaining high-pressures in the proximal and distal femur. The highest pressures are obtained with stem inserted into Palacos-R at 4 minutes 30 seconds, with proximal thumb occlusion. Collar occlusion may be better in achieving higher pressures, with lower viscosity, Simplex-P.

P37
MIGRATION OF THE ACETABULAR CUP FOLLOWING IMPACTION GRAFTING

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Following hip arthroplasty carried out using the Slooff-Ling impaction grafting technique micro-motion of the acetabular cup is frequently seen within the bone graft bed. In some cases this can lead to gross migration and rotation of the acetabular cup, resulting in failure of the arthroplasty. The movement of the cup is thought to be due to the irrecoverable deformation of bone graft under shear and compressive forces. Previous experimental studies have addressed ways in which the behaviour of the bone graft material may be improved, for example through washing and the use of improved particle size distribution. However there has been a limited amount of research carried out into assessing the behaviour of the acetabular construct in-vivo.

This study presents a 3D finite element model of the acetabular construct and hemi-pelvis following impaction grafting of a cavitory defect. A sophisticated elasto-plastic material model was developed based on research carried out by the group to describe the bone graft bed. The material model includes the non-linear stiffness response, as well as the shear and consolidation yield response of the graft. Loading associated with walking, sitting down, and standing up is applied to the model. Distinct patterns of migration and rotation are observed for the different activities. When compared in a pseudo-quantitative manner with clinical observations results were found to be similar. Walking is found to account for superior migration, and rotation in abduction of the acetabular cup, while sitting down and standing up are found to account for posterior migration, and lateral rotation. The developed 3D model can be used in the assessment of cup designs and fixation devices to reduce the rate of aseptic failure in the acetabular region.

P38
KINEMATICS OF A MEDIAL PIVOT TOTAL KNEE REPLACEMENT

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Introduction: Restoration of predictable and normal knee kinematics after a TKR can improve the patient's function. Traditional designs exhibit grossly abnormal kinematics with the femur subluxing posteriorly in extension and a paradoxical forward slide in flexion. In addition, the kinematics are very variable. Newer designs were intended to overcome these problems, owing to their ability to provide 'guided motion' of the components. The medial pivot knee uses a specifically designed articulating surface constraining the femoral component to externally rotate about an axis through the medial compartment.

This study assesses the functional in vivo kinematics of Advanced Medial Pivot (AMP) TKR and compares it to kinematics of the normal knee.

Methods: Thirteen patients with pre-operative diagnosis of primary osteoarthritis, who had undergone a knee replacement with the AMP knee at least one-year prior were recruited in this study. All had an excellent clinical outcome (as assessed by AKSS) and underwent fluoroscopic analysis whilst performing a step up activity. Knee kinematics were assessed by analysing the movement of the femur relative to the tibia using the Patella Tendon Angle (PTA) through the range of knee flexion. This data was compared to that of thirteen normal knees.

Results: The PTA for the normal knee has a linear relationship with knee flexion. The PTA is 14 degrees in full extension and decreases to -10 degrees at 100 degrees knee flexion during a step-up exercise. Between extension and 60 degrees of knee flexion, no significant difference was found between the PTA for the normal knee and for the AMP. The PTA for AMP is significantly higher for values of knee flexion exceeding 60 degrees. The standard deviation for different values of knee flexion is similar to that seen in the normal knee.

Conclusions: In extension, the PTA is near normal but in flexion PTA is higher than normal suggesting that the femur is too anterior. The variability of the kinematics for AMP TKR is similar to that of the normal knee and is better than that of most other knee designs that we have studied in the past, indicating that it is a stable TKR.

P39
THE CONTRIBUTION OF BONE CREEP TO VERTEBRAL DEFORMITY. A PRELIMINARY STUDY

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Introduction: Atraumatic vertebral deformity could possibly arise from sustained loading by the adjacent intervertebral discs, especially when discs are degenerated and press unevenly on the vertebra (1). Creep phenomena have been studied in samples of cancellous and cortical bone, but little is known about their potential to deform whole bones. We hypothesise that sustained asymmetrical loading of a vertebral body can cause differential creep, and vertebral deformity.

Materials and methods: Five thoracolumbar 'motion segments' (two vertebrae with intervening soft tissues) were dissected from human cadavers aged 64-88 yrs. Each specimen was subjected to a 1.5 kN compressive force for 2 hrs, applied via plaster moulded to its outer surfaces. Specimens were positioned in 2 deg flexion to simulate a stooped posture. Six reflective markers were attached to pins inserted into the lateral cortex of each vertebral body. Anterior, middle and posterior vertebral body heights were measured at 1 Hz to an accuracy of 7 microns, using a MacReflex 2D optical tracking device. This enabled elastic and creep strains in the vertebral cortex to be plotted against time. Compressive 'stress' acting vertically on the vertebral body was quantified by pulling a miniature pressure transducer along the midsagittal diameter of adjacent discs (1).

Results: Maximum elastic compressive strains in the posterior, middle and anterior cortex were 500-700, 800-2000 and 600-2500 microstrains respectively. Corresponding creep strains were 200-1500, 200-3200 and 500-5500 microstrains. Increased strains in the anterior vertebral body corresponded to increased stresses in the anterior annulus of adjacent discs. Creep was greater in older specimens, and was only partially reversible. 'Permanent' anterior wedging of the vertebral body could reach 0.7 deg after 2 hrs.

Discussion: These preliminary results suggest that vertebral deformity in-vivo can arise by creep mechanisms, when total (elastic+creep) strain locally exceeds the yield strain of bone (2). Future experiments will consider the middle vertebra in three-vertebra specimens.

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P40
PREDICTING THE EFFECT OF WEAR RATE REDUCTION ON THE INCIDENCE OF ASEPTIC LOOSENING

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Introduction: The concept that aseptic loosening is a function of polyethylene wear has led to the use of cross-linked polyethylene in total hip arthroplasty (THA). We studied the relationship between polyethylene wear rate and aseptic loosening in order to model the potential effects of wear-reducing strategies on the failure rate for each prosthetic component.

Methods: 350 subjects who had previously undergone Charnley THA were divided into 3 groups: Controls (n=273); those with loosening of only the femoral stem (n=43); and those with only cup loosening (n=34). Polyethylene wear was measured using a validated method (EBRA). The relationship between wear rate and loosening was examined using logistic regression analysis, and estimates of the effect of wear rate modulation made using odds-ratios.

Results: The median annual wear rate in the controls (0.07mm) was lower than both stem looseners (0.09mm, p=0.002) and cup looseners (0.18mm, p<0.001). The odds of cup loosening increased 4.7 times per standard deviation (SD) increase in wear rate above the reference (control) population (p<0.001). The odds of stem loosening increased 1.7 times per SD, but was not independent of other risk factors (p>0.05). The potential reduction in risk of loosening was calculated using the following formula: (OR^SD2)/(OR^SD1), where 1 and 2 are the new and old mean z-score wear rates. Thus, for a 25% or 50% reduction in wear rate, the incidence of cup loosening may reduce by 71% and 293%, respectively. The rate of stem loosening may, at best, reduce by 7% and 17%, respectively.

Discussion: Wear reduction strategies, such as cross-linked polyethylene, have the potential for a major impact on the incidence of cemented cup, but not stem, loosening.

P41
CLINICAL MEASUREMENT OF THE WRIST MOTION USING A THREE-DIMENSIONAL ELECTROMAGNETIC GONIOMETER

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Dynamic assessment of the wrist motion and the specific angles are difficult using the conventional methods. We wanted to adapt and assess the repeatability of the Fastrak system for continuous monitoring of three dimensional (3 D) wrist movements.

Twenty seven volunteers, aged 18 to 30 years were asked to perform predetermined tasks. The exclusion criteria were previous history of wrist trauma or joint disease. The transmitter was mounted on the dorsum of the forearm while the sensor was placed over the third metacarpal head. The protocol of three tasks was developed. Task 1 measured maximal flexion, extension, radial and ulnar deviation of the wrist. Task 2 involved picking up an object and moving it across a barrier. Task 3 involved the writing simulation. The comparison between the left and the right wrists indicated suitability of the system to be used on either of the limbs. Repeated measurements on the right wrist provided an assessment of repeatability of the Fastrak system.

The Fastrak system was successful in acquiring data in 3 D. The transmitter and the sensor were easy to attach and were of no discomfort to the subjects. As expected the maximum movement was noted in the flexion-extension plane. The total arc of movement in the flexion-extension plane was 127.1 degrees and 69.7 degrees in the radio-ulnar plane. There was no statistically significant difference between the movements in the left and the right wrists, even when the effect of dominance was considered. The lift and move task showed that most subjects utilised three-fourths of the total possible radio-ulnar movement, but only one-thirds of the total flexion and extension. The writing simulation revealed a substantial variability between subjects. The Fastrak system revealed variation up to 3 degrees in the means of range of movements, while measuring wrist movements.

The current study showed that the Fastrak system is a user-friendly and repeatable device, which could be used in everyday clinical use. It has the potential to be used for evaluation of the diseased wrist and the results of therapeutic interventions, operative or otherwise.

P42
VALIDATION OF 'STRESS' MEASUREMENTS INSIDE DEGENERATED INTERVERTEBRAL DISCS

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Introduction: 'Stress profilometry' involves pulling a pressure transducer through a loaded intervertebral discs in order to characterise the intensity of loading within it. The technique has been used to explore how stress distributions vary with age, spinal level, degeneration, creep loading, and injury. However, can the output of the strain-gauged transducer (which is calibrated in a fluid) really quantify stress perpendicular to its membrane when inserted into the fibrous matrix of degenerated discs?

Methods: Thirteen full-depth cylinders, 7mm in diameter, were cut from inner, middle and outer regions of the anterior and lateral annulus of two human upper-lumbar discs aged 74 and 82 yrs. Specimens were confined within a metal cylinder of internal diameter 7 mm. Two vertical slots on opposite sides of the metal cylinder allowed a pressure transducer, side-mounted near the tip of a 0.9 mm-diameter needle, to be pulled through the annulus sample. Constant compressive loading was applied for 20s to the top of the annulus sample, using a plane-ended 6.9 mm-diameter indenter, while the transducer was pulled through the sample. Transducer output was sampled at 25Hz. 'Stress profiles' were repeated with the transducer orientated vertically and horizontally, and with 6-21 values of compressive load, corresponding to stresses up to 3MPa. Average values of measured 'stress' were compared to applied stress (compressive force/indenter area).

Results: Measured (average) vertical compressive stress was linearly related to applied stress, with Rsq values averaging 0.97. The gradient of the line averaged 0.98 (range 0.77 - 1.28) indicating that measured stress values approximated to applied stress, and were not merely proportional to it. For horizontal measurements, the Rsq and gradient averaged 0.97 and 0.92 respectively. Abnormal results in 3/13 specimens appeared to be affected by transducer damage and were disregarded.

Conclusion: Stress profilometry can quantify compressive stress within the annulus of degenerated intervertebral discs. This fibrous tissue appears to be sufficiently deformable to allow efficient coupling of stress between the matrix and transducer membrane.

P43
CONTACT STRESS DISTRIBUTION IN REAMED HIP HEMIARTHROPLASTY, A FINITE ELEMENT MODEL

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We previously demonstrated that cartilaginous tissue was induced on a reamed acetabular articulation in an ovine hemiarthroplasty model with three different femoral head sizes. At maximum loading during stance phase, the acetabular peak stresses immediately after reaming could reach approximately 80 MPa under direct implant-bone contact with in-vitro measurements.

We aimed to establish finite element (FE) models of the ovine hip hemiarthroplasty which examine stress distribution on the reamed acetabula by three head sizes. We hypothesized that the stress distribution did not differ between different sizes when the joint is congruent and that the peak stresses in the acetabulum immediately after reaming occurred in the dorsal acetabulum.

Three two-dimensional FE models of ovine hip hemiarthroplasty were built; each comprised a head component, 25, 28, and 32 mm in diameter, and an acetabular component. The acetabular geometry was acquired from an ovine acetabular histological section. The head was moved to partly intersect with the acetabulum representing the reaming procedure and a congruent contact was confirmed. Cortical bone and cancellous bone were modelled as linear elastic, with moduli of 20 and 1.2 GPa, respectively. Variable moduli were also assessed. The finest mesh for each model consisted of over 100,000 four-node quadrilateral elements. Loading conditions were chosen to represent peak hip joint force developed during the stance phase. Stress distribution in the acetabular area in contact with the head was plotted against the articulating arc length.

The results confirmed that the stress distribution between different prosthetic head sizes in a reamed hemiarthroplasty model did not change when the joint was congruent. The peak compressive stresses occurred in the dorsal acetabulum with the 32 mm model being the highest at approximately 69 MPa, the 28 mm model at 63 MPa, and the 25 mm model at 54 MPa. An increase in the cancellous modulus and a decrease in the cortical modulus increased the peak stresses in the dorsal acetabulum.

This presents an indicative study into the effect of prosthetic femoral head sizes on the stress distribution in the acetabulum. The idealized 2-D models showed reasonable agreement when compared quantitatively with the in vitro study.

P44
THE WEAR OF PEEK-COCRMO AND CFR-PEEK-COCRMO ASSESSED ON A PIN-ON-PLATE MACHINE

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Introduction: In an attempt to prolong the lives of implantable devices, several 'new' materials are undergoing examination to determine their suitability as joint couplings. As part of a series of tests, polyetheretherketone (PEEK) against cobalt chrome molybdenum (CoCrMo) and carbon fibre reinforced-PEEK against CoCrMo were tested on a multidirectional pin-on-plate machine.

Materials and methods: The two four station pin-on-plate machines used in this study applied both reciprocation and rotational motion. Each material combination was tested individually on separate machines. Four samples of PEEK pins against CoCrMo plates were tested and eight samples (two tests) of CFR-PEEK pins against CoCrMo plates were tested. The pins were supplied by Invibio Ltd. A 40 N load was provided to each station. The lubricant used was 24.5 % bovine serum (protein content: 15 g/l) and this was heated to 37 degrees C. The wear was assessed gravimetrically and the tests each completed 2 million cycles.

Results: On average, the pin and plate wear factors were 7.37 and 0.010 x 10 -6 mm3/Nm for PEEK-CoCrMo and 0.144 and 0.011 x 10 -

6 mm3/Nm for the CFR-PEEK against CoCrMo specimens respectively. These results show the wear of the components corrected relative to the control specimens that therefore took into account the weight gain due to lubricant absorption.

Discussion: The CFR-PEEK pins gave considerably lower wear against CoCrMo than the PEEK pins. It is interesting to note that the total wear factor provided by high carbon CoCrMo pins articulating against high carbon CoCrMo plates (which is known as a low wearing material combination in hip implants) was found to be 0.84 x 10 -6 mm3/Nm (1) which is actually higher than that found in these studies for CFR-PEEK against CoCrMo tested under the same conditions.

Conclusions: CFR-PEEK articulating against CoCrMo provided much lower wear than the PEEK-CoCrMo samples. This material combination also gave lower wear than metal-on-metal samples. This, therefore, indicates that this material combination may perform well in joint applications.

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P45

EFFECT OF BONE QUALITY ON THE PRIMARY STABILITY OF ACETABULAR PRESS-FIT CUP AND THE CONTACT MECHANICS OF A METAL-ON-METAL HIP RESURFACING PROSTHESIS

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Metal-on-metal hip resurfacing arthroplasty is a conservative procedure that is becoming an increasingly popular option for young arthritic patients most likely to undergo a secondary procedure in their lifetime. The stability of the acetabular component is of particular concern in these patients who show an increased risk of failure of the cemented acetabular cups in conventional total hip replacements. The purpose of this study was to examine the initial stability of a cementless interference press-fit acetabular cup used in hip resurfacing arthroplasty and implanted into 'normal' versus poor quality bone. Also examined was the effect of the press-fit procedure on the contact mechanics at the cup-bone interface and between the cup and femoral head.

A finite element (FE) model of the DUROM resurfacing (Zimmer GmbH) was created and implanted anatomically into the hip joint, which was loaded physiologically through muscle and subtrochanteric forces. The FE models included: a line-to-line, 1mm and 2mm interference press-fit cup. Also considered were two FE models based on the 1mm press-fit cups, in which the material properties of the cancellous and cortical bone tissues were reduced by 2 and 4 times, to represent a reduction in bone quality as seen with age or disease.

Increasing the cup-bone interference resulted in a significant reduction in implant micromotion. All the press-fit models showed predicted cup-bone micromotion below 50 micrometers. This would ensure adequate initial stability and encourage secondary fixation through bone in-growth. The predicted acetabular stresses were found to increase with the amount of press-fit, however, there was no suggestion of a fracture. These stresses would further contribute to securing the cup.

Reducing the bone quality showed an increase in the predicted micromotion and increased bone strain. Micromotion was below 50 micrometers, but the predicted compressive bone stresses, necessary for additional implant fixation, was reduced. This implied that poor quality bone would provide unsuitable support medium for the implant. The bearing surface contact mechanics were little affected by the amount of press-fitting.

P46

GENERATION OF VIRTUAL MODELS OF BONE MICRO-ARCHITECTURE

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Micro level finite element models of bone have been extensively used in the literature to examine its mechanical behaviour and response to loads. Techniques used previously to create these models involved CT attenuations or images (e.g. micro-CT, MRI) of real bone samples. The computational models created using these methods could only represent the samples used in their construction and any possible variations due to factors such as anatomical site, sex, age or degree of osteoporosity cannot be included without additional sample collection and processing. This study considers the creation of virtual finite element models of trabecular bone, i.e. models that look like and mechanically behave like real trabecular bone, but are generated computationally.

The trabecular bone is anisotropic both in terms of its micro-architecture and its mechanical properties. Considerable research shows that the key determinants of the mechanical properties of bone are related to its micro-architecture. Previous studies have correlated the apparent level mechanical properties with bone mineral density (BMD), which has also been the principal means of diagnosis of osteoporosis. However, BMD alone is not sufficient to describe bone micro-architecture or its mechanical behaviour. This study uses a novel approach that employs BMD in conjunction with micro-architectural indices such as trabecular thickness, trabecular spacing and degree of anisotropy, to generate virtual micro-architectural finite element models. The approach permits generation of several models, with suitable porous structure, for the same or different levels of osteoporosity. A series of compression and shear tests are conducted, numerically, to evaluate the apparent level orthotropic elastic properties. These tests show that models generated using identical micro-architectural parameters have similar apparent level properties, thus validating this initial bone modelling algorithm. Numerical tests also clearly illustrate that poor trabecular connectivity leads to inferior mechanical behaviour even in cases where the BMD values are relatively high. The generated virtual models have a range of applications such as understanding the fracture behaviour of osteoporotic bone and examining the interaction between bone and implants.

P47

METAL LEVELS IN 'CELL SAVER' BLOOD RECOVERED DURING REVISION HIP ARTHROPLASTY

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Purpose: The aims of our study were: (i) to measure the total metal content in cell saver blood recovered during revision hip arthroplasty, (ii) to evaluate the efficacy of centrifuging and washing the recovered blood in reducing the metal content, (iii) to investigate whether transfusion of the salvaged blood resulted in a significant increase in the metal ion levels in the patients' blood in the immediate post-operative period.

Materials and methods: We analysed the levels of metallic debris and metal ions in cell saver blood in nine patients undergoing revision hip replacement. Using inductively coupled plasma mass spectrometry (ICP-MS), the levels were measured for titanium, aluminium, vanadium, chromium, cobalt, nickel and molybdenum. The metal ion levels were analysed using a dilution technique and the total metal content levels (particulate debris and ions) were analysed with a digestion technique.

Results: Significantly higher levels of metal ions and metal debris were found in the pre-processed blood compared with the processed blood (after centrifuging and washing). The ion levels in the processed blood were not high enough to cause a significant increase in the patients' immediate post-operative blood ion levels when compared with pre-operative levels.

Conclusion: There are markedly elevated levels of metal ions and particulate metal debris in the blood salvaged during revision total hip arthroplasty. The processing of the recovered blood in a commercial 'cell saver' significantly reduces the total metal load that is re-infused. Re-infusion of salvaged blood does not result in elevated metal ion levels in the immediate post-operative period.

P48

A THICK CEMENT MANTLE INCREASES EARLY MIGRATION OF IMPACTION GRAFTED FEMORAL STEMS

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Purpose: To investigate whether cement mantle thickness influence early migration of the stem after impaction grafting

Methods: Twelve artificial femora were prepared to mimic cavitary defects. After compacting morselized bone into the cavities, Exeter stems were cemented in place. By using all combinations of three sizes tamps and stems (0, 1 and 2), we created cement mantles of 0, 1, 2, 3 and 4 mm thickness. Bones with stems were placed in a testing machine and loaded cyclically to 2,500 N while measuring stem migration. Statistical analysis was by regression analysis. Outcomes were stem subsidence and retroversion, predictors were mantle thickness, tamp size and stem size.

Results: Average stem subsidence after 2500 cycles when using size 1 tamp and stem (2 mm mantle) was 0.94 mm. Cement mantle thickness significantly influenced stem subsidence (r=0.68, p=0.015). For a 0 mm mantle, subsidence was 0.59 mm and for a 4 mm mantle it was 2.54 mm. Cement mantle thickness also significantly influenced stem retroversion (r=0.62, p=0.031). Cement mantle thickness was a better predictor than tamp or stem size.

Discussion: Concern exists that inadequate cement mantles may affect stability of impaction-grafted stems. In our study, larger difference between tamps and stems gave substantially more subsidence and rotation, whereas a smaller difference reduced them. Concerns over thin mantles may have been premature.

P49

QUANTIFYING THE BEHAVIOUR OF MORSELLISED CORTICO-CANCELLOUS BONE

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Morsellised cortico-cancellous bone (MCB) is used extensively in impaction grafting procedures, such as the filling of cavitory defects on the femoral and acetabular sides during hip arthroplasty. Several experimental studies have attempted to describe the mechanical behaviour of MCB in compression and shear, and it has been found that it's properties can be improved by washing and rigorous impaction at the time of surgery. However their focus has not been on the development of constitutive models that can be used in computational simulation.

The results of serial confined compaction tests are presented and used to develop constitutive models describing the non-linear elasto-plastic behaviour of MCB, as well as its time dependent visco-elastic behaviour. It is found that the elastic modulus, E of MCB increases linearly with applied pressure, p, with E achieving a value of around 30 MPa at a pressure of around 1 MPa. The plastic behaviour of MCB can be described using a Drucker Prager Cap yield criterion, capable of describing yielding of the graft in shear and compression. The time dependent visco-elastic behaviour of MCB can be accurately modelled using a spring and dashpot model that can be numerically expressed using a fourth order Prony series. The role of impaction in reducing subsequent plastic deformation was also investigated. The developed relationships allow the constitutive modelling of MCB in finite element simulations, for example of the acetabular construct following impaction grafting. The relationships also act as a gold standard against which to compare synthetic graft and graft extender materials.

P50

PULL-OUT STRENGTH OF CEMENTING BIRMINGHAM (CEMENTLESS) CUPS: A BIOMECHANICAL STUDY

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Introduction: There has been a renewed interest in metal-on-metal bearing for total hip replacement with the benefit of a larger head size and decreased incidence of dislocation. In the revision hip scenario cementation of a polyethylene liner, for a previously compromised liner fixation mechanism into a preexisting well-fixed shell or a cage, has become an accepted method to decrease the morbidity of the procedure. Perhaps Birmingham cementless cups could be used as cemented devices in primary and revision hip surgery where a cementless cup is not possible.

Aim: To study the pull-out strength of cemented Birmingham sockets in an experimental model.

Materials and Methods: Eight Birmingham cups were cemented into wooden blocks after they were reamed to the appropriate size allowing for a 3mm cement mantle, multiple holes drilled into the reamed sockets and cement vacuum-mixed. Cable was then threaded through the holes on the rim of the cup and the wooden block was then mounted on a metal plate and secured. Linear tension was then gradually applied on the cup through the cable.

Results: The pull-out strength of the cemented Birmingham cups was higher than the failure of the cable. The tensile load to failure for the cables ranged from 3642.6 N to 4960 N with an average load of 4286.9 N.

Conclusion: The average tensile load of 4286.9 is very high compared to previous studies with cemented polyethylene and metal liners. This finding is very promising and might support clinical application in complex primary and revision total hip replacement.

P51

DETERMINATION OF INTERFACIAL FRACTURE TOUGHNESS OF BONE-CEMENT INTERFACE USING SANDWICH BRAZILIAN DISKS

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The long-term stability of total hip replacements (THRs) critically depends on the lasting integrity of the bond between the implant and the bone. Late failure in the absence of infection is known as 'aseptic loosening', a process characterised by the formation and progressive thickening of a continuous layer of fibrous tissue at the interface between the prosthesis and the bone. Aseptic loosening has been identified as the most common cause for long-term instability leading to the failure of acetabular cups. There is clearly a need to study the failure mechanisms in the acetabular fixation if the long-term stability of THR is to be significantly improved. The bonding strength in the presence of defects is measured using interfacial fracture toughness, and this information is not available currently.

In this work, interfacial fracture toughness of synthetic and bovine bone-cement interface has been studied using sandwiched Brazilian disk specimens. Experiments were carried out using a common bone cement, CMW, and polyurethane foam under selected loading angles from 0 to 25 degrees to achieve full loading conditions from tensile (mode I) to shear (mode II). Finite element analyses were carried out to obtain the solutions for strain energy release rate at a given phase angle (ratio of shear and tensile stress) associated with the experimental models. The effects of crack length on the measured interfacial fracture toughness were examined. Microscopic studies were also carried out to obtain the morphology of the fractured interfaces at selected loading angles.

The results show that both polyurethane foam and bovine cancellous bone seem to produce a similar type of interfacial failure of bone-cement interface, with cement pedicles being 'pull-out' of the pores of the foam/bone. Damage sustained by the cement pedicles seems to increase progressively as the increase of shear loading component. The measured values of fracture toughness are a function of crack length and phase angle, and are comparable with those published in the literature on cortical bone and cement interface.

The implication of these results on the assessment of fixation in acetabular replacements is discussed, particularly in the light of results from bovine cancellous bone-cement interface.

P52

COMPARISON OF SUTURE ANCHORS AND PULL-OUT SUTURES FOR TENDON ATTACHMENTS TO THE DISTAL PHALANX: A BIOMECHANICAL STUDY IN VITRO

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Three methods to reattach avulsed finger flexor tendons to the distal phalanx were compared: a 1.8 mm metal barbed suture anchor, twin 1.3 mm PLA (polylactic acid)absorbable anchors, or a pull-out suture over a button. The suture-anchor interface was tested by pulling the suture at 0, 45, and 90 degrees to the anchor's axis. The anchors were tested similarly in plastic foam bone substitute. Repairs of transected tendons in cadaveric fingers were loaded cyclically, then to failure. The suture failed prematurely if pulled across the axis of the anchor. Conversely, fixation in bone substitute was stronger when pulling at an angle from the axis. Cyclic loads caused significantly more gap formation in-vitro with twin 1.3 mm absorbable anchors than the

other methods; this method was significantly weaker. The 1.8 mm anchor gave similar performance to the pull-out suture over button technique, while the twin 1.3 mm absorbable anchors were weaker and vulnerable to gap formation even with passive motion alone.

P53
LESS INVASIVE STABILISATION SYSTEM (LISS PLATE) FOR THE MANAGEMENT OF PERIPROSTHETIC FEMORAL FRACTURES AROUND HIP ARTHROPLASTY

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Background: To analyse the effectiveness and complications of Less Invasive Stabilisation System (LISS plate) in the management of peri-prosthetic femoral fractures.

Materials and methods: We present a study of 18 periprosthetic femoral fractures around hip arthroplasty (16 females and 2 male patients) treated with LISS plate between September 2001 to February 2005. The average age of the patients was 81.6 years. Twelve patients had significant co-morbidities pre-operatively. All the fractures were classified according to the Vancouver classification for Peri-prosthetic fracture of femur. Ten were classified as type B1, two as type B2 and six as type C. Eleven fractures were around total hip replacement and seven were around hemi-arthroplasty (four cemented and 3 uncemented). Partial weight bearing started early post-operatively. Full weight bearing varied between 5-6 weeks depending on clinical and radiological status.The patients were followed up untill fracture union.

Results: Three patients died during the follow-up period owing to unrelated causes. The average follow up period was 11.7 months. All the remaining fifteen patients had satisfactory fracture union although one patient required further LISS plate following a fall 17 days postoperatively and another one patient developed low grade deep infection with a chronic sinus. It was noted that in one patient, plate had lifted off the bone at the proximal end with no loss of reduction of the fracture. Three patients were noted to have mild to moderate discomfort around the prominent implant. No implant breakage noted.

Conclusions: Even though LISS plate was originally designed for distal femoral fracture treatment, it appears to be very promising device in the treatment of peri-prosthetic femoral fractures (Type B1, Type C and medically unfit patients with Type B2 for stem- revision) with osteoporotic bone in elderly patients. Early mobilization is a key feature. This system involves minimally invasive approach, stable construct without need for primary bone grafting.

P54
PROOXIDATIVE AND ANTIOXIDATIVE PROPERTIES OF ORTHOPAEDIC MATERIALS

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The intensification of free radical processes at total joint replacements is well known. Wear particle-induced inflammatory reaction and metal corrosion is associated with generation of the oxygen radicals. At the normal functioning of joint implants there is a natural deterioration and constant updating of their surfaces. In these conditions probably also formation of free radicals during tribochemical reactions.

The radical-generating ability of the wear particles of orthopaedic alloys, alumina ceramics and antioxidant properties of various cured cements and UHMWPE were studied using the model reaction of cumene oxidation. Artificial wear particles of different alloys and ceramics were obtained using dry friction of a ball against a disk made of appropriate materials. Cement powders were obtained by grinding cement samples in a ceramic mortar.

Wear particles of orthopaedic alloys were found to initiate cumene oxidation whereas ceramic particles were inert. It was revealed that cobalt-chromium-molybdenum particles were much more active than titanium-aluminum-vanadium and stainless steel particles. Different amounts of antioxidants (from 2.3 to 12 millimole/kg) were detected in cured cements which considerably exceeded their amounts in the initial liquid cement components. The content of antioxidants in cured ÑÌW-1 cement was 3-5 times more than that in Palacos P and Sulcem1 cements. The amount of antioxidants was considerably lower in UHMWPE than in the mentioned cements. The reactivity of combinations of different particles is determined by relative particles' contributions, and such mixtures are able to demonstrate either antioxidative (alloy-cement mixture) or prooxidative (alloy-UHMWPE mixture) properties. In particular, cement particles suppressed cumene oxidation caused by cobalt alloy particles. Inhibition duration depends on the ratio between alloy and cement particles and on the content of antioxidants in cements. Polyethylene particles were not able to inhibit cumene chain oxidation caused by cobalt alloy particles.

Investigation of prooxidant and antioxidant behavior of the wear particles of orthopaedic materials provides better insight into their action on surrounding tissues and implant components. In particular, it is necessary to develop methods of preclinical testing that can simulate and estimate the action of radical intermediates generated in the course of tribochemical reactions on implant components.

P55
MEASURING THE SURFACE GEOMETRY OF TENDON

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More than 100,000 anterior cruciate ligament reconstructions are performed annually in the USA. The hamstrings and the patellar tendons are the most frequently used graft tissues. Up to ten percent of these grafts are deemed to have failed, generating considerable discussion in the literature regarding the ideal graft choice. A three-dimensional computational model, taking into account both material and geometrical non-linearities, would be useful in predicting the performance of different graft tissues and fixations. Unfortunately, the mechanical characteristics and parameters needed for such a model are complex and largely unknown. The aim of this study is to develop a method for measuring the geometrical properties needed as input for a three-dimensional tendon model.

A laser-based, non-contact technique is used to generate a series of cross-sectional profiles along the length of the tendon. Unlike previously proposed methods, it is able to detect concavities and can be constructed using equipment commonly found in an engineering laboratory. A laser line generator (Stocker-Yale Lasiris SNF, Quebec, Canada) projects a horizontal line onto the sample. Images of the line are acquired with a digital video camera (Basler A631fc, Germany) as the tendon is rotated. These images are reassembled into 2-D slices using MatLab software. Multiple cross-sections can be combined to create three dimensional geometries.

The new method was validated on objects of known shape (circular and hexagonal cylinders). The cross-sectional area measurement was found to be accurate to within 2.5%. The method was repeatable to within 1.7%. Six bovine flexor tendons have been analysed; concavities were evident in four of these. This method could be adapted to determine the surface geometries of other long and slender objects.

P56
FAILURE OF BONE-CEMENT INTERFACE IN CEMENTED ACETABULAR SOCKETS UNDER FATIGUE LOADING CONDITIONS

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Retrieval studies based on revision operations at King Edwards VII Hospital reveal that, although microcracks develop in the cement mantle, it is the debonding between cement and bone that often defines the final failure of cemented acetabular replacements. This was illustrated at the revision surgeries by the easy removal of the acetabular cups with cement mostly attached to the cup. It is felt that a fundamental understanding of the mechanisms that initiate and propagate the interfacial failure at the bone-cement interface is the key towards solving the problem.

In this work, in-vitro fatigue tests were carried out on cemented acetabular replacements using third-generation of composite pelvic bones. Standard Charnley cups were implanted using common bone cement, CMW, following the standard surgical procedures. The implanted hemi-pelvic bone model was then constrained at the sacro-iliac and pubic joints to represent the anatomic constraint conditions. Cyclic loads representing the maximum range of the hip

contact force during normal walking were used and the direction of the maximum hip contact force was achieved by using angled plates. In addition to standard cup position, open cup and retroverted cup positions were also examined to assess the significance of cup orientation under fatigue loading conditions.

Damage development in the reconstruction was monitored using CT scanning at regular intervals. Permanent records were collected and the sample was eventually sectioned and polished for microscopic studies. Results show excellent correlations between the results from the CT images and the microscopic studies, indicating progressive bone-cement interfacial failure in the posterior-superior quadrant.

The significance of the work in the studies of 'aseptic loosening' will be discussed.

P57
THE CREEP BEHAVIOUR OF LEADING CEMENT BRANDS

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The visco-elastic behaviour of cement, is a key feature of cement-implant performance in total hip arthroplasty.

The aim of this study was to describe the creep behaviour of the leading plain bone cements under standardised physiological in-vitro conditions.

Methods: Cements were mixed under vacuum conditions as per manufacturers instructions. Moulds were used to to produce beams of standard dimensions. These were stored in saline at 37oC for 21 days to ensure thorough polymerisation. Under the same conditions, the beams were tested for 72 hours in a 12-station quasi-static creep rig, using a four-point bending configuration. The rig applied a constant stress of 8MPa to each beam and the deflection was recorded at 8-minute intervals by a data-logging device. The porosity was measured in the mid-cross section of each beam sample using a digital image technique.

The cements tested were Palacos R, CMW1 and Smartset GHV and Surgical Simplex P.

All data were analysed using ANOVA with Bonferroni post-hoc test (SPSS).

Results: Palacos R exhibited the highest mean deflection at 72 hours (0.86+/- 0.21mm) followed by Surgical Simplex P (0.85 +/- 0.18mm), CMW1 (0.72 +/- 0.09mm) and Smartset GHV (0.60 +/- 0.16mm).The difference between the two DePuy cements and Palacos R (p=0.03) and Surgical Simplex P (p=0.04) were statistically significant. None of the beams failed during the test. The creep behaviour correlated with the cross-sectional porosity measurements.

Conclusions: This study has shown that there are significant differences in the creep bahaviour of the leading medium and high viscosity bone cements. In particular Palacos R and Surgical Simplex P demonstrate 'High' creep and the DePuy cements 'Low' creep. Creep appears sensitive to subtle changes in the composition of the material. This may be reflected in the clinical behaviour of different bone cements and stresses the importance of the time-dependent properties of PMMA.

P58
THE BIODEGRADABLE IMPLANT LITAR IN PURULENT ORTHOPEDICS

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Restoration of the bone defects on the background of the purulent osteomyelitis process is one of most pressing problems in orthopedics. In the last few years the medical procedure was improved thanks to use of semisynthetic or syntetic implantation biodegradable composite materials.

The object of the investigation is to study possibilities of use of fast-biodegradable implant LitAr (Russia) for filling infected bone defects in course of complex treating various osteomyelites forms.

The composite material LitAr (in plates) is a mixture of components: xenocollagen and hydroxoapatite. Material is intended for stimulating osteogenesis. In event of infection materials LitAr in 7-10 days is lysed by the wound and microbe ferments and cannot support purulent process. Composite material was introduced into osteomyelitis defect intraoperative through an open wound by introducing a dry substance through fistulas as well as in form of a suspension in 0.9%-sodium chloride solution. For 13.6% of patients postoperative time period was complicated by suppuration of operative wound. It was stated in course of use of material LitAr that in spite of secondary wound suppuration active osteogenesis rate was little different from similar process for patients with wound healing by first intention. It made it possible to use material more active for patients of advanced years because it was impossible to use a radical sanitation of purulent bone cavity for these patients. Material LitAr was used for 13 patients with osteomyelitis cavities. In form of a suspension (injectionally or through a fistular duct) in 0.9% NaCl solution material was introduced through fistulas for 8 patients with an affected shin bone. Roentgenological signs of consolidation emerged by 35-40 days. A complete ossification set in by 95-120 days. Immobilization was performed by use of plaster. In far-off time periods (about 2 years) no pathologic fractures were noted. 2 patients had a relapse of fistulas formation (15.4%).

The use of implant LitAr for filling infected bone defects for stimulating osteogenesis and for restoring bone continuity in a complex treatment of various forms of osteomyelitis can be considered as an effective one including for patients because it was impossible to perform a radical sequestrectomy for these patients.

P59
THERMOGRAPHIC INVESTIGATION OF DRILLING OF BONE

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Thermonecrosis either results in bone loss which may weaken the purchase of surgically-inserted screws leading to loosening or the dead bone may remain in situ and become infected resulting in a ring sequestrum. The aim of this project was to measure the heat generated during drilling of bone. By using a novel realtime thermal camera the thermal events could be visualised topographically.

An experimental setup comprising a force table, an infrared camera, a power drill and a new surgical 2.5mm drill bit was constructed. This enabled measurements of the force applied and temperature changes in sheep cortical bone during a drilling operation. The temperature was observed throughout the drilling period and for further 15s after the drill bit was withdrawn. Images were grabbed using a LAND FTI Mv thermal camera which was driven by LIPS Mini software. Calibration was made in the range 20-200 degrees C, the upper value being provided by a high wattage resistor. Data was processed using routines written in MATLAB.

It was found that 12s were required to drill through a single cortex. Within one second of drilling, the maximum recorded temperature in the vicinity of the drill increased from the baseline of 20 to 170 degrees C. It remained above this temperature for 25s. Immediately after the drill bit was withdrawn, a region of approximately 15mm of diameter of cortical surface had a sustained temperature above 50 degrees C. After 15s of cooling, this diameter had only reduced to 10mm. By modelling the cooling curve, the maximum temperature at the drill tip was extrapolated to be between 500-600 degrees C.

Thermography has proven to be useful in the study of the thermal characteristics of bone during drilling. The process of drilling generates significant increase in temperature in the vicinity of the drill. This temperature elevation has been found to be sustained for a significant period of time.

P60
ANATOMICAL AND MORPHOLOGICAL VARIATIONS IN LUMBAR SPINE IN CHILDREN WITH OSTEOGENESIS IMPERFECTA

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Introduction: Lumbar spine morphology is well described in healthy children but has not been described in children with Osteogenesis Imperfecta (OI).

Aims: To look at lumbar bony morphometry in OI children and to consider the importance of these factors in spinal surgery in these children

Methods: 21 lumbar vertebrae (from L3-5) of 7 OI (6 OI type 3 and 1 OI type 4) children with scoliosis were analysed using Reformatted

Computer Tomographic scans. The following measurements obtained: Spinal canal diameters, Transverse pedicle width, Total pedicle length, Pedicle root length, Transverse pedicle angle and Sagittal pedicle angle. Results are compared with previously published data of normal age-matched lumbar spine measurements

Results: The mean age was 12 years (range 7-18 years). 6 females and 1 male. All had spondylolisthesis at L5-S1.Results were analysed by Wilcoxon Signed Rank test (nonparametric test). The transverse pedicle width was significantly narrower at all 3 levels ($p<0.01$). Transverse pedicle angle was significantly less angled at all 3 levels (L3 $p=0.04$, L4 & L5 $p<0.01$) whilst the sagittal pedicle angle was significantly more angled at all 3 levels ($p<0.01$). Spinal canal diameter (AP) was significantly increased at all 3 levels (L3 & L5 $p<0.01$, L4 $p=0.02$). And no significant differences in spinal canal transverse diameter and total pedicle length. Pedicle root length Significantly longer at all 3 levels (L3 & L4 $p<0.05$, L5 $p<0.01$). All children had grade-I spondylolisthesis at L5/S1.

Conclusions: A longer pedicle root with a narrower transverse diameter (and thinner cortices) and a reduced transverse angle is essential knowledge when passing pedicle screws in the lumbar spine in children with OI. This is a difficult technique and its safety requires further evaluation

P61

NOVEL APPLICATION OF PQCT TO STANDARDISE SYNOVIAL FLUID BIOMARKER CONCENTRATIONS

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Objective: To challenge the validity of using biomarker concentrations in synovial fluid for the assessment of joint pathology.

Hypothesis: Synovial fluid biomarker concentrations are influenced by both cartilage and synovial fluid volumes.

Methods: Synovial fluid volumes were determined from the equine metacarpophalangeal (MCP), proximal interphalangeal (PIP) and distal interphalangeal (DIP) joints, which have different disease prevalences.

Chondrocyte density was calculated from a defined site in each joint.

Cartilage volume was measured by novel application of Peripheral Quantitative Computed Tomography (pQCT).

Cartilage oligomeric matrix protein (COMP), glycosaminoglycans (GAG) and total protein (TP) concentrations were measured and then adjusted for cartilage and synovial fluid volume and compared between joints.

Results: Mean synovial fluid volume was significantly greater in the MCP than the distal joints ($p<0.0001$) (3.2 ±0.5ml, 0.5 ±0.1ml and 0.6 ±0.1ml respectively). In contrast, the DIP had the greatest cartilage volume compared to the proximal joints (5360 ±667mm3 2640mm3, 1940 ±331mm3 respectively). There was no significant difference in the cartilage cellularity between all joints.

The DIP had higher TP, COMP and GAG concentrations, however, when values were expressed per unit cartilage volume the opposite was found, with the MCP then exhibiting significantly higher concentrations.

Conclusions: These data show the joint with the highest prevalence to osteoarthritis has the lowest biomarker synovial fluid concentrations but the highest biomarker levels per unit cartilage, suggesting a higher release. These results indicate that meaningful interpretation of biomarkers in synovial fluid require consideration of both fluid and cartilage volume.

P62

HIP FRACTURES IN NONAGENERIAN PATIENTS-OUTCOME OF SURGICAL TREATMENT

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Background: Nonagenarian patients with hip fractures present many challenges to the clinician, both in terms of their advanced age and medical co-morbidities with potential orthopaedic complications. Our aims were to assess outcome of hip fractures in a nonagenarian population with respect to pre-operative predictors of outcome, immediate and long-term morbidity, and survival rates.

Methods: All nonagenarian patients with a hip fracture admitted to our unit between January 2000 and December 2003 were considered. Eighty-one patients were included, the majority being female (M: F 14: 67). Ages ranged from 90 to 98 years for female patients (mean 92.5 years, SD 2.2) compared to 90 to 95 years for male patients (mean 92.7 years, SD 2).

Results: Delay to surgery was 1.25 days and the median ASA grade was III. The method of anaesthesia used was spinal in 78% and general in 22%. The majority of patients had intertrochanteric fractures and methods of fixation involved internal fixation in 63% and hemiarthroplasty in the remaining 36% of the group deemed fit for surgery. The rate of complications during inpatient stay was 19% and there were eight in-patient post-operative mortalities due to medical complications. Mean survival post hip fracture in our patient group was 474.7 days (median 372.5 days). Within forty days of surgery 25% of patients died, including our inpatient mortality of 10%. However, 50% of the patients were still alive 126 days post-operatively.

Conclusion: Hip fractures must be given special attention in the nonagenarian population because of their advanced age and medical co-morbidities. Careful pre-operative assessment and medical maximisation combined with prompt surgical intervention yielded a good outcome and return to pre-injury status for most patients. Lower ASA grades, surgery within 48 hours, and increased pre-operative haemoglobin levels were all associated with favourable outcomes. Medical complications were the major cause of morbidity and mortality with a low rate of orthopaedic complications. The majority of patients were able to return to their previous residence and continued to be mobile with various levels of assistance.

P63

MEDIUM TERM FOLLOW UP OF THE PIPINO COLLUM FEMORIS PRESERVING TOTAL HIP ARTHROPLASTY

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A prospective study to review 75 consecutive Pipino collum femoris preserving total hip arthroplasties (CFP THR): using the Harris Hip Score (HHS) to assess functional outcome in the short and medium terms.

The Pipino prosthesis was introduced as an alternative to hip resurfacing because of its bone preserving capability. Preserving the femoral neck to a greater extent saves valuable bonestock for possible revision procedures. The stem (proximal 2/3) and acetabular cup are hydroxyapatite coated. Bearings were all either ceramic or metal on polyethylene. All procedures were performed or directly supervised by the senior author.

Patients in the cohort were assessed pre-operatively, in the short term and the medium term using the Harris Hip Score(HHS). Hip radiographs were performed at medium term follow-up to assess for radiological signs of aseptic loosening.

The study is based on a cohort of 70 patients, 34 male and 36 female with mean age of 52 (range 13-71).Followed up over a mean period of 43 months (range 17-60). 70 patients were contacted and 64 patients were reviewed. Four patients were lost to follow-up. Indications for surgery were Osteoarthritis (56); Rheumatoid arthritis (8); AVN (3); SUFE (2); Perthes (2); DDH (1); Psoriatic Arthropathy (1).

The cohort's preoperative HHS showed a mean 50.1 (range 25-88). This increased to a mean of 95.9 (range 55-100) in the short term review period, during the medium term review the mean 93.6 (range 63-100).With 82% of patients in the excellent group and 88% good to excellent group.

At the final review there was one case of aseptic loosening (Cup) which required revision surgery. There were 2 dislocations and one intraoperative lateral femoral wall fracture and no cases of superficial or deep infection.

In conclusion we believe that the Pipino collum femoris preserving total hip arthroplasty has excellent short and medium term results.

P64

RELATIONSHIP BETWEEN BONE MINERAL DENSITY AND RATE OF FUNCTIONAL RECOVERY AFTER DISTAL RADIAL FRACTURE

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Older fracture patients frequently ask whether their osteoporosis will affect fracture healing. There is only limited previous data about this. We investigated recovery after distal radial fracture, and compared it with BMD of the other distal radius and the lumbar spine (measured using quantitative CT).

All 28 patients had sustained a dorsally displaced distal radial fracture which was deemed to require treatment by intrafocal wire fixation. All patients had acceptable correction of dorsal and radial angle at final x ray (3 months). Wrist function was measured using the Patient Rated Wrist Evaluation (PRWE - a validated outcome measure for use after distal radial fractures), grip strength,and range of motion. All measurements were made at 6, 12 and 26 weeks. BMD was measured in the opposite wrist and the lumbar spine using QCT at 6 weeks after fracture.

There was no correlation between recovery of grip strength (% of contralateral grip strength) at 6,12,or 26 weeks with BMD at either site. Similarly, there was no correlation between BMD and either absolute PRWE scores at any time point or improvement in PRWE between time points. The strongest predictor of recovery of grip appeared to be the proportion of grip recovered at 6 weeks (correlation between% grip recovered at 6 weeks and 3 months r = 0.85; at 6 weeks and 6 months r= 0.56; both p <0.001). This was not affected by age or variations in measured final dorsal or radial angles or length within this group. It was not affected by degree of preoperative fracture displacement.

These data suggest that recovery of function after distal radial fractures is not influenced by osteoporosis. The data about the importance of initial recovery of grip suggest that factors other than bone position and bone healing may affect rate of functional recovery after distal radial fracture.

P65

AO VOLAR PLATE FIXATION FOR UNSTABLE OR COMMINUTED DISPLACED DISTAL RADIUS FRACTURES

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Purpose: Management of the unstable or comminuted displaced fractures of the distal radius is difficult. We report our experience treating these fractures with AO volar plate fixation. An attempt to introduce a new radiological classification for the accuracy of surgical reduction is made. The classification includes 10 criteria and 100 points.

Methods: 124 patients had volar plate fixation performed between June 2000 and May 2003 using AO volar plate. We reviewed clinically and radiologically 101 patients; 60 were type C and 41 were type A (after failed conservative treatment). The average follow up is 37 months (24 - 57). The average age is 46 years (19 - 81). Postoperative regimen consisted of immediate physiotherapy and a wrist splint for three weeks. Cooney's modification of Green and O'Brien and Sarmiento's modification of Gartland and Werley were used for clinical assessment. Lidstorm and Frykman used for radiological assessment.

Results: At final follow up the means of distal radius parameters were: volar tilt of 9 degrees, radial inclination of 22 degrees, radial height is 11mm and palmer cortical angle of 32 degrees. The mean dorsiflexion was 61 degrees, palmer flexion was 59 degrees, pronation was 80 degrees and supination was 76 degrees. Grip strength was 86 percent of the opposite side. The average DASH score was 13.6. There was 14 poor results, 6 of them had significant loss the initial reduction. There was significant correlation between our classification and the clinical outcome.

Conclusion: AO volar plate fixation of unstable distal radius fractures provides strong fixation that maintains reduction and allows early mobilisation.

P66

TURNING PARTIAL KNEE REPLACEMENT 'INSIDE OUT': THE KINEMATICS OF THE DOMED LATERAL OXFORD UNICOMPARTMENTAL KNEE REPLACEMENT

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Background: The Oxford unicompartmental knee replacement (UKR) use in the lateral compartment has been associated with a reduced flexion range, increased medial compartment pain and a higher dislocation rate than seen with its medial counterpart due to the inadequacy of a flat tibial tray replacing the domed anatomy of the lateral tibia. A new design incorporating a domed tibial component and a biconcave meniscal bearing has been developed to overcome these problems. This current study was designed to establish whether this modified 'domed' implant has maintained the established normal kinematic profile of the Oxford UKR.

Methods: The study population consisted of 60 participants from three equal groups; Group 1- Normal volunteer knees (n = 20), Group 2 - Flat Oxford Lateral UKR's (n = 20) and Group 3 - Domed Oxford Lateral UKR's (n = 20). The sagittal plane kinematics of each involved knee was assessed continuously using videofluoroscopic analysis. A standardised protocol of step-up and deep lunge was used to assess loadbearing range of motion during which the patella tendon angle (PTA) was measured as a function of the knee flexion angle (KFA).

Results: PTA/KFA values compared at 10 degree KFA increments from maximal extension to maximal flexion for all 3 groups did not demonstrate any statistically significant difference in PTA values between any group as measured by a 3-way ANOVA. The Domed implant achieved higher maximal active flexion during the lunge exercise than those with a Flat implant. Only 33% of the Flat UKR's achieved KFA of 130 degrees or more under load whilst performing a lunge, compared with 75% of domed UKR's and 90% of normal knees. No Flat UKR achieved a KFA of 140 degrees or more, yet 50% of all domed UKR's did, as also did 60% of all normal knees.

Conclusions: There is no significant difference in the sagittal plane kinematics of the domed and flat Oxford UKR's. Both implant designs have a favourable kinematic profile closely resembling the normal knee. The domed knees though do have a greater range of motion under load as compared to the flats, approaching levels seen with the normal knee.

P67

TREATMENT OF ATROPHIC FRACTURE NON-UNION WITH THE SYNTHETIC COMPOUND TP508

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Over 1 million fractures occur each year in the UK. Approximately 5-10% of these fractures have problems with healing. The treatments used for these patients often have a poor outcome and are associated with increased morbidity and disability. Application of synthetic peptides such as thrombin degradation peptide (TP508) has been shown to accelerate fracture repair in a closed rat femoral fracture model. Controlled release of TP508 using microspheres has been shown to enhance repair of articular cartilage defects and stimulate bone formation in segmental defects in rabbits. The aim of this study was to determine whether TP508 could bring about healing in an established fracture non-union model.

A validated rat model of fracture non-union was used. The model was created and left for 8 weeks in order to represent a clinically equivalent model of a non union of a fracture. Rats were randomised into two treatment groups receiving 10microg and 1microg doses of TP508 diluted in 50microL of microspheres and delivered directly to the non union site using percutaneous injection 8 weeks after surgery. The control group received no treatment. At 16 weeks post-surgery, osseous bridging was assessed both radiographically and histologically.

Radiographically there was no difference between the control and two treatment groups. However, histomorphometric analysis demonstrated that bone formation increased by 43.9% in animals that received high dose of TP508 compared to the control animals. The analysis also indicated that administration of the low dose of

TP508 increased the amount of bone formation compared to the control by 9.9 %.

Administration of TP508 has been shown to enhance healing of segmental defects in both critically and non-critically sized defects. However, in our model which is an established fracture non-union model, TP508 did not manage to achieve full osseous union. It has been suggested that the action of this peptide is concentration and environment dependent possibly indicating that TP508 might be less effective when administered in a chronic situation such as that associated with the established non-union fracture. However, even in this sub-optimal situation an increased amount of bone formation was observed.

P68

MEASUREMENT AND CLINICAL SIGNIFICANCE OF HYALURONAN LEVEL IN SYNOVIAL FLUID OF THE KNEES

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To explore the relationship of hyaluronan level in synovial fluid of the knee with the degree of synovitis and cartilage injury.

A total of 104 knees in 102 patients with knee osteoarthritis or other knee diseases was studied. The hyaluronan level in the synovial fluid of the knees was measured with enzyme linked immunoassay. The pathology of the synovium and articular cartilage was evaluated with Ayral's score system and Outerbridge's score system under arthroscopy. The data were analyzed by t'-test or nonparametric test, ANOVA, Pearson or Spearman correlation and multiple liner regression.

The results showed that the hyaluronan level in the synovial fluid of the knees was correlated positively with Ayral's score (beta'A=0.497, P<0.001) and negatively with accumulative Outerbridge's score (beta'O=-0.364, P<0.001), especially Ayral's synovitis

score in 104 cases. The hyaluronan level in the synovial fluid of the knees was higher in those with Ayral's score > and = 60 than in those with the score<60 (P<0.001). The hyaluronan level in the synovial fluid of the knees was lower in those with accumulative Outerbridge's score > and = 10 than in those with the score < 10 (P<0.05). The level of hyaluronan in the synovial fluid in the knees with Ayral's score > and = 60 was correlated negatively with accumulative Outerbridge's score (beta'O=-0.437, P<0.001) and positively with Ayral's score (beta'A=0.339, P<0.01), especially accumulative Outerbridge's score.

Compared with other knee diseases, the hyaluronan level of OA knees was lower (P<0.05). However, Ayral's score and accumulative Outerbridge's score were higher in OA knees (P<0.001).

The hyaluronan level in the synovial fluid of the knee can reflect the degree of synovitis and accumulative cartilage injury, especially synovitis. It reflects the degree of accumulative cartilage injury mainly when synovitis is more severe. The decrease of the hyaluronan level in the synovial fluid of OA knee is results of integrating effect of the synovitis and cartilage injury.

P69

FALLS IN PATIENTS WITH HIP AND KNEE OSTEOARTHRITIS: THE IMPACT OF JOINT REPLACEMENT SURGERY

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Background: Falls are a major concern in the elderly population both from a clinical perspective and that of health resource provision. This study evaluates the incidence of falls in patients awaiting hip or knee replacement and the impact of joint replacement surgery 2 years later.

Method: Patients aged 65-80 years listed for primary hip or knee arthroplasty for osteoarthritis (OA) were invited to participate. Patients completed a questionnaire including Western Ontario and McMaster University OA Index (WOMAC) scores 0-100, 100 best, history of falls and fractures. Function was measured using Timed Up and Go (TUG) walk test. All tests were repeated at two years.

Results: One hundred and ninety-nine patients (84 hips, 115 knees) were recruited with a mean age of 72 years (standard deviation 4.0) and predominantly female (57 %). At two years 144 patients were reviewed of whom 128 had undergone arthroplasty. After surgery, 29/128 (23%) reported falling compared to 55 of these 128 (43%) falling at baseline; only 13/128 (11%) had fallen more than once. Fifteen patients sustained minor injuries and one patient reported a fractured wrist. Of the patients who had undergone joint replacement and fell at baseline 36/55 (66%) patients reported no falls at follow-up, whilst there were 11 new fallers. Patients reporting falls had significantly lower WOMAC pain and function scores, and slower TUG scores at both baseline and two-year review.

Conclusion: Patients with severe hip and knee OA awaiting arthroplasty reported a higher incidence of falls compared to the normal population but reported fewer falls after surgery. However, almost one in four patients were still reporting falling at the two-year review. Injury including periprosthetic fractures can have serious clinical and economic consequences. This study highlights the need to evaluate a falls prevention programme in arthroplasty management.

P70

HOW CAN ORTHOPAEDIC SURGEONS' REFERRAL RATES FOR DEXA BE IMPROVED FOR PATIENTS AT HIGH RISK OF OSTEOPOROSIS?

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Background and objective: in 2003 in its publication 'Care of fragility fracture patients' The British Orthopaedic Association highlighted the orthopaedic surgeon's role in assessment and management of patients at high risk from osteoporosis. In general such secondary prevention of osteoporosis is carried out poorly by orthopaedic surgeons. This audit aimed to determine if software which identifies patients at high risk from osteoporosis from clinic letters, improves orthopaedic surgeons' referral rates for DEXA.

Methods: two audit cycles were carried out using local guidelines. The audits concerned patients over 50 years having sustained a fragility fracture of the distal radius. According to local guidelines all such patients should undergo DEXA. Patients were identified from hospital records and the number referred for DEXA determined. Those who had undergone DEXA in the year prior to fracture were excluded. The baseline audit was from April to June 2004 inclusive followed by closure of the loop between October and December 2004 following reinforcement of guidelines. Following continued poor referral rates at this point the software programme was introduced. It identifies patient age and key words in dictated clinic letters when they are being printed, for example distal radial fracture. Appropriate patients have computer generated osteoporosis advice included at the bottom of the general practitioner letter along with a DEXA referral form which General Practitioners complete. A further audit using similar methods was carried out 3 months after the software introduction (January 2006).

Results: baseline audit identified forty-three patients (36 women and 7 men) with a mean age of 73 years, 3 were referred for DEXA (7%). Following reinforcement of guidelines fifty-two patients were identified (46 women and 6 men) with a mean age of 68 years, 16 (31%) were referred. At re-audit (following the introduction of the software programme) 45 patients were identified (38 women and 7 men) with an average age of 71 years. 30 (67%) were referred for DEXA. This is a significant improvement using a Chi squared analysis.

Conclusion: the software programme significantly improves orthopaedic surgeon identification of patients at high risk of osteoporosis and referral rates for DEXA.

P71

A PROSPECTIVE STUDY OF KNEE LAXITY AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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Aims: To assess the results of Anterior Cruciate Ligament (ACL) reconstruction at a minimum of two years follow-up, using the Rolimeter [AIRCAST, Europe] as an adjunct to routine knee examination and subjective scoring systems.

Methods: The Warrington Knee Injury database was initiated in June 2001 and data from all knee ligament injuries has been collected prospectively, from preoperative status through to all follow up assessments. Inclusion criteria for our study were, all ACL reconstructions performed by the senior author with minimum 24

months follow up; other ligaments being intact and presence of a normal contralateral knee.

50 patients satisfied the inclusion criteria. There were 41 males and 9 females in ages ranging from 17 to 51 (mean 30.6 years), with no significant difference in age between sexes. Hamstring grafts were used in 29 knees and Bone-Patellar tendon - Bone (BPTB) grafts in 21. Knee laxity was measured using the Rolimeter with IKDC knee examination and functional assessments using the Lysholm, IKDC and KOOS scoring systems.

Results: 20/21 of patients with BPTB grafts (95.2%) and 26/29 of patients with Hamstring grafts (89.7%) achieved normal or near normal knee laxity compared to their opposite knee. The Range of movement in 48 of 50 knees (96%) fell within normal or near normal limits according to IKDC description (Lack of extension < 3 degrees and lack of flexion < 10 degrees). Two patients with abnormal range of movement had a similar lack of movement preoperatively. Though none of the knees were abnormally tight (AP laxity difference <-3), there was a relationship between knee tightness and lack of extension, but this was not statistically significant. There was no association between age or sex of patient and lack of movement. The mean IKDC, Lysholm and KOOS symptom scores were 80.45, 87.3 and 81.3 respectively.

Conclusions: We have achieved a normal or near normal AP laxity in 92% of our ACL reconstructions on assessment at 2 years postoperatively. We report no significant difference in outcome between use of Hamstring or BPTB grafts. The functional outcome has been optimal as revealed by subjective evaluation.

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ELECTROMYOGRAPHIC ASSESSMENT OF FOREARM MUSCLES IN LATERAL EPICONDYLITIS

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Purpose: It is known from the literature that gripping, which is commonly used in various work-related, sport-related, and daily activities, activates both wrist extensors and flexors. Pain aggravation occurs during grip due to over-exertion of the extensor muscle group in lateral epicondylitis and grip strength is reduced. Of grip strength studies, few studies have simultaneously investigated muscular response using electromyography as a method of monitoring muscular fatigue or muscular activity of forearm muscles. The fatigability and activity of wrist antagonistic muscles in patients with lateral epicondylitis has not been previously investigated.

Methods: 16 tennis elbow patients (Tennis Elbow Group) and 16 healthy volunteers (Control Group) were participated in this study. In both groups, local muscular fatigue and muscular activity were measured for 3 forearm muscles contributing to the wrist extension and 2 muscles contributing to the wrist flexion using EMG and during gripping at 50% maximum voluntary contraction (MVC). Fatigability and activity of muscles then were compared between control and tennis elbow groups.

Results: Grip strength was significantly lower in tennis elbow group than that in control group ($p < 0.05$). Median frequency (MDF) and root mean square (RMS) of electromyographic signals were used as parameters to measure muscular fatigue and muscular activity, respectively. Further analysis showed no significant difference in the fatigability of forearm muscles between two groups. The activity of Extensor Carpi Radialis (ECR) showed statistically significant reduction in tennis elbow group compared to the control group ($p < 0.05$).

Conclusion: This is the first study to simultaneously investigate the fatigability and activity of the forearm antagonistic muscle groups in patients with lateral epicondylitis. The fact that ECR showed similar level of muscular fatigue to other muscles despite decreased muscular activity may indicate of higher fatigability of this muscle in tennis elbow. Furethermore, decreased muscular activity of ECR may be a part of mechanism to protect the muscle from further injury in tennis elbow patients.

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Abstract withdrawn

P74

PRINCIPAL COMPONENT ANALYSIS OF DISTAL RADIAL FRACTURE KINEMATICS DURING CYCLIC ACTIVITIES OF DAILY LIVING

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Aims: To detect discriminant features in the cyclic kinematic patterns generated during selected upper limb activities of daily living by a normative and a distal radial fracture group, so as to reduce the multidimensionality of the kinematic analysis.

Methods: Cyclic activities of daily living were performed using a protocol that allowed comparison between the resulting kinematic patterns or waveforms. Twop groups were measured:

Group A: 11 subjects with normal hand function (average age: 31.5ys, SD: 8.7ys).

Group B: 5 subjects having undergone treatment for distal radial fracture was tested using the same methods (average age: 34.2ys, SD: 16.8ys).

Task presented here, (one of 5) performed by turning a key 90 degrees clockwise. Principal component analysis (PCA) was applied to the waveforms of group A, using the procedure illustrated by Deluzio et al.,1997 for use with walking gait patterns. A 90% trace criterion was used to calculate the number of principal components (PCs) to retain.

Results: Looking at elbow pronation/supination (PS). Two PCs were retained. The first component consisted of a simple pronation pattern. The opposite signs of Y1 differentiated left-hand users (utmost right), who required pronation to rotate the key, from right-hand ones, who required supination, with the exception of subject 3 group B. The second component consisted of pronation (cycle first half) followed by supination (second half). Subject 3 stood out because of limited elbow supination, which resulted from the combination of pronation (Y1) and supination (Y2) components.

Conclusions: Upper limb analysis can employ the statistic tools of gait analysis provided a cyclic and repeatable protocol is used. PCA was applied to elbow PS to identify statistically different movements of the distal radial fracture group and underline their main characteristics. This is particularly important in the presence of a large data group, when the identification and evaluation processes need to be both rapid and accurate. Limited PS was identified as a discriminant feature, supporting the follow-up studies for this injury that measured a reduction of PS by about 80% compared to that of the unaffected side. The cycle stages concerned can be identified on the basis of the contribution given by each component.

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INVESTIGATION OF RADIO-LUNATE RELATIONS IN NORMAL AND FRACTURED WRISTS

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Restoration of normal anatomy following a distal radial fracture is an important factor in determining functional recovery. However, current methods of assessing dorsal tilt and displacement require 'true' lateral radiographs, and important reference points are often obscured by metalwork.

Aims: to investigate if an easily identifiable and predictable relationship exists in the normal wrist between the distal radius and lunate; and if so,to compare fractured wrists (pre and postoperatively)using conventional and new assessment methods.

22 patients with displaced distal radial fractures treated by ORIF, were included. Patients had pre and postoperative radiographs taken of the injured and uninjured wrists. From lateral radiographs, measurements were performed using the PACS system. A line was superimposed upon the dorsal radial cortex 2cm proximal to the wrist passing distally. The following measurements were performed: lunate height, distance from the 'line' to the superior and inferior poles of the lunate, and conventional measurements of dorsal tilt and angulation.

Uninjured wrist: Most noticeably the dorsal radial line always passed superior to the lunate, mean distance of 3.27mm (1.75-6.6mm). As a ratio, the distance from the line to the superior pole of the lunate divided by the distance to the inferior pole ('lunate ratio') had a mean of 0.16 (0.11-0.19).

Fractured wrist, PreORIF: Using conventional methods, the mean fracture displacement was 2.64mm (0-5.1mm) and the mean dorsal tilt was 23.3 degrees(4 degrees volar tilt to 43 degrees dorsal tilt). Using the dorsal reference 'line', in all cases the lunate was either above or transected by the line; mean lunate ratio of 1.61 (0.54-8.05). The mean height of the lunate projecting dorsal to the line was 9.5mm (6.1-16.1mm).

Fractured wrist, PostORIF: Apart from one radiograph, the 'line' passed superior to the lunate; mean distance of 2.64mm (0-3.9mm), with a mean lunate ratio of 1.13 (0.61-2.74). These measurements correlated well with measurements of dorsal tilt and displacement.

Our study suggests that there is a strong relationship between the distal radius and the lunate that could be used to assess fracture displacement and quality of reduction. Its main advantages are simplicity and ease of use despite the presence of metalwork.

P76

DOES FLUOROSCOPY WITH THE MINI C ARM RESULT IN DECREASED RADIATION EXPOSURE COMPARED TO THE CONVENTIONAL C ARM IN EXTREMITY ORTHOPAEDIC SURGEON?

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Introduction: The mini C arm is a surgeon operated fluoroscopic device for use in the operating theatre for extremity orthopaedic surgery. There have been no studies comparing the radiation dose of the mini C arm and the conventional C arm.

The aim of this study was to determine if the exposure to patient and surgeon was decreased with use of the mini C arm.

Methods: This was a case-control study. Operations performed with the mini C arm were matched for type, complexity and operator with cases performed with the conventional C arm. The number of exposures and the total time of exposure were measured, and the skin dose and scatter calculated.

Results: There were 16 case-control pairs. There was a significantly greater number of exposures taken by the surgeon operated mini C arm (p=0.02), but there was still a significantly lower exposure to the surgeon with the mini C arm (p=0.004). There was no significant difference in the patient skin dose (p=0.21).

Conclusions: The surgeon operated mini C arm results in a greater exposure time and number of exposures. Despite this, the mini C arm exposes the surgeon to less radiation compared to the conventional C arm in extremity orthopaedics. The radiation exposure with the mini C arm is approximately half that of the conventional C arm. The increased number of exposures may occur because surgeons are more trigger happy with the mini C arm, or because there are technical problems with achieving a useful image. The mini C arm should be used for extremity orthopaedics whenever possible to decrease the radiation exposure.

P77

DOES INTRA-ARTICULAR STEROID INCREASE THE INFECTION RATE FOLLOWING HIP ARTHROPLASTY?

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Intra-articular steroid injection has been widely used for relief of pain in Osteoarthritis. Recent studies show an increasing rate of infection in these patients following hip arthroplasty. We have reviewed our cohort of patients to see if they are susceptible to higher infection rate.

We reviewed a cohort of 167 consecutive hips that had at least one injection with a 40mg triamcinolone acetonide and 4ml 0.5% bupivacaine mixture to relieve the symptoms of hip osteoarthritis or to clarify a diagnosis of hip arthritis between January 1997 and November 2004 were reviewed. A total of 37 hips (36 patients) that subsequently proceeded to have a total hip arthroplasty were selected as our study group. There was a minimum of a one-year follow up.

The rate of infection in our initial cohort of patients following a hip injection was 0.60% (1 hip) which resulted in repeated washouts and a subsequent total hip arthroplasty with a good outcome. On review of the 37 hips, one was revised due to a deep infection secondary to staphylococcus epidermidis. Four were revised for continued instability and pain with no evidence of infection either prior to or during revision. When deep infection is taken as an endpoint, cumulative survival at 7.5 years is 0.968 (95% confidence interval of 1 to 0.905). The total survivorship of this cohort if all revisions are included is 0.852 at 7.5 years (95% confidence interval of 0.730 to 0.974). The revision rate due to a deep infection in our study is 2.7%.

We conclude that patients who have a total hip arthroplasty after a hip injection do not have an adversely high rate of deep infection.

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ACCURACY OF CLINICAL EXAMINATION OF THE KNEE IN ACUTE INJURIES:A PROSPECTIVE CORRELATION WITH ARTHROSCOPIC FINDINGS

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With the increasing availability of magnetic resonance imaging, there is potentially less emphasis being placed on making a definitive clinical diagnosis. Changes in the undergraduate curriculum have also reduced the emphasis on orthopaedic clinical evaluation. This aim of this study was to evaluate the predictability of clinical examination alone in comparison with arthroscopic findings in 50 consecutive patients presenting for arthroscopy to our service. Four trainees examined each patient; each examiner was blinded to the clinical diagnosis made by their colleagues. All patients were examined in the ward and subsequently underwent examination under anaesthesia and arthroscopy.

Of the tests for meniscal injuries joint line tenderness was the most sensitive (77%) and specific (68%). Apley's and McMurray's test while specific (92%, 98%) lacked sensitivity (9%, 30%). Overall the tests for anterior cruciate ligament (ACL) disruption were more reliable than the tests for meniscal injuries. The anterior drawer and Lachmann tests had high specificity (90%, 75%) and sensitivity. The pivot shift test also had very high specificity (75%) and sensitivity (98%) for detecting ACL injuries. These data demonstrate that joint line tenderness is the most reliable sign of meniscal injury. In the absence of joint line tenderness Apley & McMurray's tests have little role in routine clinical examination. Clinical tests and signs of ACL deficiency are consistently reliable in diagnosing ACL rupture.

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HOW RELIABLE ARE PRINTED DIGITAL RADIOGRAPHS FOR USE IN PRE-OPERATIVE ARTHROPLASTY TEMPLATING?

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Aim: to compare the reliability of pre-operative templating for total hip and knee arthroplasty using printed digital radiographs versus conventional radiographs.

Materials and Methods: a prospective continuous study commenced January 2005. The PACS digital imaging system was introduced in May 2005 and the radiology department adopted a policy of printing orthopaedic radiographs to 'true size'. All consultants and their registrars undertaking primary total hip and knee arthroplasty were asked to participate in the study and agreed. The operating surgeon completed a proforma for each Total Hip Replacement (THR) performed noting the templated cup and stem size and offset. Following the surgery the actual sizes and offset of the components implanted were also recorded on the proforma. A similar procedure was followed for the femoral and tibial components of Total Knee Replacements (TKR).

Results: there were 254 completed proformae. 186 proformae for conventional radiographs and 68 proformae for printed digital radiographs. Templating was possible from all the conventional radiographs; however templating was only possible from 58 of 68 (85%) digital radiographs as the images were obviously not true size. The templated sizes of both hip and knee components from conventional radiographs were more predictive of the actual size implanted in all cases. Furthermore there were a greater number of predicted outlying sizes using printed digital radiographs.

Conclusion: digital radiographs, even those said to be true size are unreliable for the purposes of pre-operative planning.

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EARLY TREND IN FAILURES DUE TO FRACTURE NECK OF FEMUR IN BIRMINGHAM RESURFACING HIP ARTHROPLASTY: MINIMUM OF FIVE YEARS FOLLOW-UP RESULTS FROM AN INDEPENDENT OUTCOME CENTRE

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The Trent arthroplasty register reported that results of Hip arthroplasty in general setup were less than that reported from specialist centres by 5%. This independent prospective study tests the hypothesis that results of Birmingham Hip Resurfacing (BHR) arthroplasty from pioneering centres would not accurately represent the outcome of hip resurfacing when performed in general setup.

All patients were prospectively followed for at least five years at Oswestry Hip outcome centre. The surgeons carrying out the operation prospectively provided surgical details and thereafter patients were followed using Oswestry hip questionnaire (OSHIP) at fixed intervals. Survival was assessed by Kaplan-Meier method. Results were compared to the published results of BHR from specialist centres .

There were 679 patients, and 58 surgeons in the study. Mean age at operation was 51 years and mean follow up was 5.63 years. The predominant preoperative diagnosis was Osteoarthritis. Mean OSHIP score was 89.5. There were 29 (4.2%) failures mostly due to fracture neck of femur (34%). Out of 14 failures in the first year, 9 (64%) were due to fracture neck of femur. The Kaplan-Meier survival up to eight years is 95.354% in the current study.

Compared to the published results, there were 2 to 19 times high failure rate which is significantly higher (p=0.001) than the published studies. Most of the early failures were due to fracture neck of femur in the first year. Hence we prove our hypothesis, as the results of BHR from specialist centres do not accurately reflect on the outcome in general setup. The discrepancy in the results is mostly due to fracture neck of femur in the early postoperative time. The results of this study will enhance awareness of the early trend in failures. Appropriate patient selection and meticulous surgical technique will help avoid this complication in the general setup, where most of the patients get benefited from BHR arthroplasty.

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TIBITALOCALCANEAL ARTHRODESIS IN NEGLECTED PAINFUL ANKLE WITH RETROGRADE INTRAMEDULLARY VERSA NAIL

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The authors report the use of a modified 'Y-V' medial capsular repair in association with Scarf osteotomy for Hallux valgus in 55 patients (62 feet) aged 18 to 61 years (mean 43 years) between July 2004 and July 2005. All patients were followed up for minimum 6 months by questionnaire, physical examination (American Orthopaedic Foot and Ankle Society score) and comparison of preoperative and post operative x rays.

Using this technique none of the patients required an additional proximal phalangeal osteotomy (Akin Osteotomy). At six months follow up American Orthopaedic Foot and Ankle Society score improved from 46 to 87. Intermetatarsal (IM) angle and the hallux valgus (HV) angle improved from 16 degree to 9 degree and from 31 degree to 16 degrees respectively (p less than 0.05).

Of the sixty two procedures 59 did not develop any complications. Two had superficial infections which required oral antibiotics only. One partial loss of correction of hallux valgus occurred for which the patient refused a second operation. Seven cases had some residual pronation deformity of the big toe identified by the patients who felt the deformity was 'about 50%' compared to before the operation.

Akins osteotomy achieves an apparent correction of hallux valgus without addressing subluxation of metatarso-phalangeal joint. Our technique reduces the metatarso-phalangeal joint and corrects the hallux valgus angle anatomically.

We recommend the use of this modified 'Y-V' medial capsular repair to correct the hallux valgus angle and reduce the need for an additional procedure to augment the correction achieved during Scarf osteotomy for hallux valgus.

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POSTOPERATIVE HYPOKALEMIA: ITS INCIDENCE, CAUSES AND IMPLICATIONS FOR ELDERLY PATIENTS WITH FRACTURE NECK OF FEMUR

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Aims: Hypokalemia is a common electrolyte imbalance with signficent effects. The aim of our study is to identify incidence, causes and prognostic implications of postoperative hypokalemia in elderly patients operated for fracture neck of femur.

Methods and material: Retrospective study, of 404 consecutive fracture neck of femur patients who were operated in our hospital between October 2001 and July 2003. Patients identified with postoperative hypokalemia the medical notes, fluid charts and anaesthetic notes were analysed for age, preoperative morbidities, medications, mechanism and type of injury, waiting time for operation, pre, peri and postoperative fluid management, type of anaesthesia, operative time, hospital stay and mortality.

Results: Out of the 404 patients, 54(13.3%) were hypokalemic (K<3.5mmol/l) postoperatively. Of the 54 patients 16 (29.6%)had preoperative hypokalemia.

Among the hypokalemic group the mean preoperative potassium was 3.69mmol/l and the mean postoperative potassium was 3.19mmol/l. The t-test showed a statistically significant difference between mean pre and postoperative potassium levels

(P< 0.0001). High association was found with hypokalemia and post-operative dextrose infusion (38%). 50% of patients on diuretics developed hypokalemia post operatively. Interestingly, only 18% of these were hypokalemic pre operatively.

In patients with multiple medical problems, like diabetes, hypertension and CVA, high incidence of hypokalemia was found. (38% had 2 or more medical problems).

No significance in the mortality rate was found in fracture neck of femur patients with and without postoperative hypokalemia (40% vs. 39% at 3yrs).

Conclusion: There is significant risk of hypokalemia following orthopaedic surgery, especially in the elderly. This avoidable condition, which has serious consequences, should be dealt with care in the orthopaedic units. Fluid infusion regimes and should be formulated and medications reviewed to prevent conditions like hypokalemia.

P83

POSITION OF CIRCUMFERENTIAL SUTURE AND CORE SUTURE KNOTS IN TENDON REPAIR AND ITS INFLUENCE ON GAPPING AND FAILURE ENERGY

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Purpose: To find if there is any difference in gapping of tendon repair on cylcic loading and energy to failure of tendon repair when the circumferential suture knot is placed on the same side of the core suture knot or to the opposite side of the core suture knot.

Methods: Ten pig flexor tendons were repaired using 3 0 braided Polyester (Ethibond) as core suture (modified Kessler) and 6 0 Nylon as circumferential stitch (Halstead). Five tendons were repaired with the circumferential suture knot placed on the same side as the core suture (Group I) and the other five placed on the opposite side (Group II). Allocation to either of the groups was random. Using a testing machine the tendons were cyclically loaded and the energy to failure was calculated. Gapping during cylical loading was recorded using digital images.

Results: Mean gapping in Group I was 0.01 mm and in Group II was 0.03mm. This was not statistically significant (2x3 ANOVA, $p > 0.3$). The mean load to failure in group I was 58.7 N (55 to 65) and in group II was 59.5 N (54 to 67). This was also not statistically significant ($p > 0.3$ one way ANOVA).

Conclusion: There is no difference in gapping and energy to failure of tendon repair when the circumferential suture knot is placed on the same side of the core suture knot or to the opposite side of the core suture knot.

P84

POSTERIOR STABILISED TKA: IS THE CAM-POST MECHANISM EFFECTIVE?

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Introduction: The cam-post mechanism of Posterior Stabilized Total Knee Arthroplasty (PS-TKA) should provide a constraint that limits anterior translation of the femur on the tibia in flexion and thereby ensure femoral roll-back with progressive knee flexion. In a previous fluoroscopic study we showed that the sagittal plane kinematics of a PCL substituting TKA (Scorpio PS) was abnormal in flexion, suggesting inefficiency of the cam-post mechanism. We also assessed the movement of the femur relative to the tibia using the Patella Tendon Angle (PTA) through the range of knee flexion (0 to 90 degrees). The aim of the current study was to investigate in greater detail why the cam-post mechanism was ineffective by assessing the contact point movement and the distance between the cam and post.

Method: Twelve patients with Scorpio PS TKA underwent fluoroscopic assessment of the knee during a step up exercise and a weight bearing deep knee bend. The image distortion was corrected using a global correction method and the data was analysed using a 3D model fitting technique. Having determined the component position, the minimum distance between cam and post were determined. The femoro-tibial contact positions of the medial and lateral condyles were determined relative to the mid-coronal plane of the tibial component. The PTA was calculated by measuring the angle subtended by patella tendon with the tibial axis and was plotted against knee flexion angle (KFA).

Results: The relationship between PTA and KFA was abnormal relative to the normal knee. Between extension and 60 degrees flexion there was forward movement of both medial (11 mm) and lateral (5 mm) femoral condyles. Thereafter, both condyles moved back (10 mm). The cam-post mechanism failed to engage in one case while in others it engaged between 70 to 100 degrees.

Conclusions: The 3D analysis has confirmed the preliminary findings of the previous study using the PTA and KFA relationship. Despite the cam engaging in flexion normal knee kinematics were not restored. The femoral roll-back is inadequate and starts to occur at least 20 degrees before the cam and post engage.

P85

CASE FINDING OF OSTEOPOROSIS PATIENTS FROM AN ORTHOPAEDIC THEATRE DATABASE

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Many osteoporosis units are now identifying low impact fracture patients at presentation and assessing them for osteoporosis risk using a nurse led fracture liaison service (FLS); we established such a service in July 2002. Unfortunately many patients previously admitted with hip fractures have never been assessed, but are at high risk of future fracture. Outlined below is an audit of case finding using the theatre database to identify these patients.

All fractured neck of femur cases from 1999 to 2002 were identified on a theatre excel database. We utilised our Hospital Information Services System (HISS) to exclude those who had subsequently died. Current address and other personal/GP details were also found using HISS. Patients under 80 years of age received a questionnaire on osteoporosis risk factors, treatment and subsequent fractures and were invited for a Dexa scan.

Results: 675 patients were identified, of which 291 (43%) died. We were unable to obtain details on 74 (11%) patients. 96 patients under 80 years were invited for a Dexa. 45/96 replied to the letter, 36/45 agreed to a scan. 9/45 declined. Only 6/96 had a scan from GP previously & 8 had been commenced on therapy since fracture (3: Calcium/D3 supplements, 4: bisphosphonates and 1: both). 32 had a Dexa following the audit (M/F - 9:23). 4 (11%) did not attend. 21 (65%) were osteoporotic, of which only 2 were taking bisphosphonates & 1 calcium/D3. 11 (34%) were osteopenic of which 1 was on Calcium/D3 and 10 had no treatment.

Although this is quite a labour intensive intervention, it did identify many untreated osteoporotic patients who were a high risk of future fracture. It also highlighted the small number of patients who are referred for Dexa or commenced on treatment by their GP following the fragility fracture. We would recommend this strategy to other units for case finding. This emphasizes the importance of a FLS and the need to have active ways to implement NICE guidance.

P86

ASSESMENT OF HEALING IN DISTRACTION OSTEOGENESIS IN PATIENTS WITH AN ILIZAROV FRAME

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The Ilizarov frame is a circular external fixator, invented by Professor Ilizarov in Siberia during the 1950's. It uses the principle of distraction osteogenesis to form new bone in a variety of clinical situations where bone lengthening or realignment is needed. The Ilizarov frame began to be used in western medicine during the 1980's and by 1993 over 6000 cases had been performed in Europe.

Plain x-ray is one of the methods used to monitor the progress of patients fitted with an ilizarov frame.

The aim of this study is establish a pattern of healing over time in patients with the Ilizarov frame using plain x-ray films. This will improve understanding of the procedure, aid clinicians in deciding when frame removal is appropriate and provide a method of early detection should healing not be progressing appropriately.

This is a retrospective study looking at a series of 58 digitised anterior-posterior x-ray films of the tibia and fibula, taken at set time points post-operatively, from 17 patients fitted with an ilizarov frame (19 separate legs with ilizarov frames in total). Image J, an image analysis system, was used to measure pixel density from vertical slices down the centre of each fracture gap and at set intervals horizontally across the fracture gap. A mean pixel density value for each fracture gap was also calculated. The x-rays were standardised using a standard step wedge.

Promising preliminary results show pixel density to be greater towards the medial aspect of the tibia, but this difference in pixel value decreases with time. This suggests that calcification of the new bone occurs medially to laterally across the tibia. Full results will be available in April and aim to build a picture of the fracture gap at set time points post-operatively, showing a pattern of calcification in patients with the Ilizarov frame that will become a useful clinical tool for deciding time of frame removal as well as affording early knowledge of problems with the healing process.

P87

PERIACETABULAR OSTEOTOMY: EXPERIENCE IN A NON-SUPER-SPECIALIZED CENTER

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The purpose of this study was to review the early results of a consecutive series of patients undergoing periacetabular osteotomy (PAO) at Cappagh National Orthopaedic Hospital. The procedure was first carried out in 1998, and a total of 85 PAOs have been performed in 79 patients. The mean follow-up was 42 months (range 6-84 months). There were 72 females and 7 males with a mean age at the time of the operation of 22.9 years (range, 14-41 years).The preoperative diagnosis was developmental hip dysplasia in 80 hips, Legg-Calve-Perthes disease in one hip, congenital coxa vara in three hips, and slipped capital femoral epiphysis in one hip. The average Merle d'Aubigne score increased from 12.4 points preoperatively to 16 points at latest followup. The lateral center edge angle of Wiberg was between - 20 and +28 before surgery and was improved from 12 to 48 (average 30 degrees) following PAO. While, the anterior center edge angle of Lequesne and de Seze was between - 22 and +35 preoperatively and was improved by an average of 28 degrees (range, 17 - 40) postoperatively. The acetabular index angle decreased from an average of 24.8 preoperatively to 8.4 postoperatively. Clinical follow-up revealed that 77% of patients had no or mild pain, 33% of patients had a limp and 64% of patients were unlimited in physical activity, representing a markedly improved clinical outcome. Four patients underwent subsequent total hip arthroplasty. The short term results in this group of patients treated with PAO show reliable radiographic correction of deformity and improved clinical scores. The study reflects the learning curve associated with performing this procedure and the results that can be expected with a smaller clinical case-load than described in previous

studies. We suggest that PAO may safely be carried out at a non-super-specialized institution provided the surgeons have sufficient experience and patients are selected appropriately.

P88

ARE EXISTING POLYETHYLENE TIBIAL INSERT SELECTIONS FOR TKA ADEQUATE? AN EXPERIMENTAL STUDY EVALUATING SENSITIVITY OF SOFT TISSUE TENSION TO INSERT THICKNESS

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Introduction: Optimal soft tissue tension maximises function after total knee arthroplasty (TKA). Excessive tension may lead to stiffness and or pain, while inadequate tension can lead to instability. Composite component thickness is a prime determinant of this soft tissue tension. The variable component thickness provided by polyethylene inserts generally allows for 2-3 mm incremental change. This study analyses the effect of incremental change in polyethylene thickness on soft tissue tension.

Methodology: Computer assisted (Stryker Knee Nav) TKA was performed on 8 cadaveric knee specimens (4 pairs). Kinematic data was collected through the navigation software. The soft tissue tension was analysed by measuring compartmental loads. A validated load cell instrumented tibial insert was used to measure medial and lateral compartmental loads independently. The effect of 1mm increments in polyethylene thickness on compartmental loads was evaluated.

Results: We measured an increase in compartmental loads with increasing insert thickness. However the peak loads in each compartment showed different behaviour reflecting varying tension in the medial and lateral sides. The peak loads generated also showed a reduction after reaching a maximal level with further increase in insert thickness. With a 1 mm increase in insert thickness, 50 % of specimens showed greater than 200 % increase in the peak loads in the lateral compartment.

Conclusions: The compartmental loads vary as a function of insert thickness. The high sensitivity of compartmental loads with a 1mm increment is significant and has not been previously appreciated, especially intraoperatively. The currently available TKA inserts with 2-3 mm increments may make obtaining optimal soft tissue tension difficult. In addition to the current focus of obtaining accurate leg alignment, further computer aided techniques are required to address soft tissue tension.

P89

THE QUALITY OF LIFE OF YOUNG PATIENTS FOLLOWING HIP FRACTURES

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The aim of the study was to measure the Quality of Life of young patients sustained fracture neck of femur.

This is a retrospective study of 50 patients who suffered different types of hip fractures. 67 patients underwent surgeries for hip fractures between 1998-2002 were sent the 'EUROQOL EQ-5D' questionnaire, out of which 50 replied back. Different parameters of EQ-5D including mobility, self-care, usual activities, pain / discomfort, anxiety / depression and the overall health status were graded by these patients. The overall scores were calculated .we compared those values with calculated EQ-5D values of control groups of the same age from the general population of the UK. Information about The type of fractures, the type of operation, complications, and the mechanism of injury were recorded and their effect on the quality of life was correlated.

Out of 50 patients, there were 29 male and 21 female, with a mean age of 48.52 yrs (16 to 60 yrs). There were 32 patients with intra-capsular neck of femur fractures, (16 undisplaced & 16 displaced) 17 intertrochanteric and one with a subtrochanteric extension. 16 patients underwent Internal fixation (AO Screws), 15 had a hemiarthroplasty, 18 had Dynamic hip screws and 1 had THR. The mean hospital stay was 7.14 days (3 to 28 days). 70% of the patients reported some problems with mobility, 44% had problems in self-care, 58% had a restriction of their usual activities, and 70% had pain & discomfort at an average of 4 years of follow up. When compared with same age groups from the general population there was statistically significant difference in the EQ-5D index and EQ-5D state with p value of [p=<. 05] in the patient aged between 30 and 60.and no statistical differences between the EQ-5D index or EQ-5D state in the age groups between 20 and 39. We also found an association between poor life quality and development of complications.

Complications included one dislocated hemiarhtroplasty, one patient had AO screws removed.

Conclusions

We concluded that fracture neck of femur in young patient lead to significant deterioration in patient quality of life when compared with the same age groups from he general population. More research is required to improve the current treatment methods.

P90

ASSESSMENT OF UPPER LIMB MUSCULAR STRENGTH IN TENNIS ELBOW

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Purpose: It is known from previous studies that reduced grip strength is associated with tennis elbow; however; assessment of muscular strength over other parts of upper limb, particularly wrist and shoulder, has received a little or no attention in the literature. To address possible other upper extremity muscular strength weakness-imbalances in Tennis Elbow, this study aimed to investigate the strength of various upper limb muscle groups in tennis elbow patients and compare them with those of healthy subjects.

Methods: A total of 32 participants were assigned into two groups of Control (N=16) and Tennis Elbow (N=16). In both groups, upper limb maximal isometric muscular of dominant and non-dominant sides was measured at various joints including metacarpophalangeal (extension & flexion), wrist extension & flexion), grip, and shoulder (internal and external rotation and abduction) using appropriate either commercial or purpose-built dynamometers. Muscular strength and important strength ratios were analyzed and compared in each group (dominant vs non-dominant) and also between Control and Tennis Elbow group using various statistical methods.

Results: Significant dominance difference was found in all strength measurements for Control group but not for Tennis Elbow group indicating a generalized and widespread upper limb muscular weakness associated with tennis elbow. In addition, significant differences were found not only for various hand strength measurements but also for shoulder strength between Control and Tennis Elbow groups ($p < 0.05$).

Conclusion: This is the most comprehensive study of upper limb isometric muscular strength assessment in Tennis Elbow during recent years. Distributed upper limb muscle strength weakness exists in Tennis Elbow which needs to be addressed within both preventative and treatment strategies.

P91

THE RISK OF TRANSMISSION OF INFECTION FROM FRESH FROZEN ALLOGRAFT BONE

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Femoral head allograft bone used in complex orthopaedic surgery may transmit infection from donor to recipient. In order to minimise the risk all donors are serologically screened for Hepatitis B and C, HIV, HTLV, and syphilis at the time of donation and again at 6 months post-donation. Culture swabs are taken from the acetabulum and femoral head for 48 hour anaerobic and aerobic culture, and a sample of bone is incubated for 5 days in enrichment broth culture.

We have audited the culture results and screening tests performed in our bone bank from 2000 to 2005 inclusive.

1,528 allografts were received of which we had to discard 52 (3.4%) because of either positive cultures or serology. The vast majority of the positive cultures were due to S. epidermidis (30/43). All cultures were bacteria one might expect to find as normal skin flora. 3 patients had positive hepatitis C serology and 6 were syphilis EIA positive.

In May 2004 we decided in line with National Transfusion Guidelines for blood donation, to exclude donors who had had a blood transfusion since 1980 to minimise the risk of transmission of CJD.

This and the opening of an Independent Treatment Centre (ITC) in our area drastically limited the number of possible donors to our bone bank. There was a significant reduction in the number of femoral heads received in 2004 and 2005 when compared with years 2000-2003 (p = <0.00001).

We conclude that negligible numbers of femoral head allografts are lost due to our serological and microbiological screening tests. However measures introduced to limit the theoretical transmission of CJD via a bone allograft and the opening of a local ITC have had a huge impact on the number of potential donors available to us. To date the CJD prion has not been isolated from bone, but there have been 3 reported cases of transmission of infection by blood transfusion. We fear that the imminent introduction of a serological test for CJD will limit the number of possible bone donors even further.

P92

IS BONE AGE USEFUL IN PREDICTING CONTRALATERAL SLIP IN SCFE?

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Introduction: Prophylactic pinning of an asymptomatic hip in SCFE is controversial. Bone age has been used as evidence of future contralateral slip risk and used as an indication for such intervention. The efficacy of bone age assessment at predicting contralateral slip was tested in this study.

Patients and Methods: 18 Caucasian children prospectively had bone age assessment using wrist and hand x-rays when presenting with a unilateral SCFE. Patients and parents were informed about the chance of contralateral slip and risks of prophylactic fixation, and advised to attend hospital immediately on development of symptoms in contralateral hip. After in-situ fixation of the affected side prospective monitoring in outpatient department was performed. Surgical intervention was undertaken if the contralateral hip was symptomatic.

Results: Three children (2 boys) went on to develop to a contralateral slip at a mean of 20 months from initial presentation. 6 children (5 boys) were deemed at risk of contralateral slip due to a bone age below 12.5 years for boys and 10.5 for girls. Only one from this group developed a contralateral slip. The relative risk of proceeding to contralateral slip when the bone age is below the designated values was 1 (95% confidence interval of 0.1118 to 8.95). The sensitivity and specificity were 33% and 66% respectively. With positive predictive value of 15% and diagnostic efficiency of 61%.

Conclusion: Delayed bone age by itself is not a good predictor of future contralateral slip at initial presentation. Routine prophylactic pinning is not justified based on bone age alone, with the risks of surgical fixation it carries. Prospective long term longitudinal study is required.

P93

COMPARATIVE MORPHOMETRY OF PORCINE AND HUMAN THORACOLUMBAR VERTBRAE

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The growing interest in the development of spinal implants has led to an increasing need for biomechanical studies. Porcine spines are commonly used in such studies. Quantitative data of the normal porcine thoracolumbar spine is lacking, yet these data are crucial to discussion of such studies. In this study we aim to provide such a database to highlight the differences between the porcine and human specimen with a view to help plan future studies contemplating their use.

6 adult (18-24 month old, 60-80 kilograms) male porcine spines were dissected of soft tissue. The lowest thoracic and all the lumbar vertebrae were studied (n=42). 15 anatomical parameters from each vertebra were measured by 2 independent observers using digital calipers (Draper PVC150D, accuracy ± 0.03mm). The mean, SD and SEM were calculated using Microsoft Excel. Results were compared with available data on human vertebra (Zindrick et al 1987;Panjabi et al 1991,1992; Kumar et al 2000).

The inter class correlation coefficient for the observers was 0.997. The intra-observer agreement was statistically robust (0.994). The vertebral body height of the porcine vertebra was larger while both the upper and lower endplate depth and width were smaller than the human specimens. The pedicle width and depth was greater than the human specimen. The spinal canal length and depth of the porcine spine were smaller than humans indicating a narrow spinal canal. The spinous process length showed an increase from T16 to L1. This was in contrast to human spinous process. The results for the measured parameters and their comparison to human specimen will be presented.

Results from our study provides a database of anatomical measurements for the porcine vertebrae and highlights the differences with the human specimen. The data would help design future studies contemplating the use of pig spines. Biomechanical studies involving interbody cages, disc replacements and pedicle screw systems should take into account the differences and match implant size accordingly. It also provides valuable information for geometric and Finite Element Modelling of the porcine spine. Further, the results are useful in extrapolation of data from experiments which have used the porcine model.

P94

BREAST RECONSTRUCTION SURGERY USING LATISSIMUS DORSI FLAP: DOES IT AFFECT THE SHOULDER FUNCTION?

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The purpose of this study was to assess shoulder function after breast reconstruction surgery using latissimus dorsi flap.

Sixty-eight patients (72 breasts) had this operation. Average follow up was 38 months (range 24 to 54 months). DASH and Constant-Murley were used for clinical assessment. Twenty-nine shoulders found to have a normal function; whereas, 11 shoulders had mild disability, 10 shoulders had moderate disability and 8 shoulders had severe disability. However, only 6 patients reported being unsatisfied with their outcome. Furthermore, all these 6 patients were not satisfied with their breast reconstruction outcome.

This study confirms that following breast reconstruction surgery using latissimus dorsi flap, there is a considerable deterioration of shoulder function of varying degrees. Nevertheless, shoulder function is not the main concern of this group of patients.

P95

INDICATORS OF REHABILITATION IN LOWER LIMB ARTHROPLASTY

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Introduction and Aims: Allogenic blood transfusion is often required in lower-limb arthroplasty. The aims of this study were (1) to analyze the influence of anaemia on post-operative fatigue, hand grip strength, duration of in-patient physiotherapy and post-operative morbidity score (POMS) and (2) to investigate for prognostic factors to predict functional recovery following primary arthroplasty of the lower limb.

Patients and methods: This study was approved by the regional ethics committee. Two hundred patients (88 THR, 99 TKR and 13 hip resurfacing) were evaluated in this prospective trial. Blood haemoglobin concentration (Hb), hand grip strength and vigour scores using a validated fatigue questionnaire were estimated both preoperatively and at 3 days following surgery. Postoperative morbidity score (POMS) and the required duration of in-patient physiotherapy were also noted. The protocol for blood transfusion was for those with Hb less than 8 g/dL and/or post-operative symptoms attributable to anaemia.

Results: A greater fall in postoperative Hb correlated significantly with a greater reduction in post-operative vigour score (p=0.02). Also a greater fall in vigour score was found to correlate significantly with the duration of in-patient physiotherapy (p<0.001). A reduction in Hb of >4g/dL from the pre-operative Hb predicted a significantly higher reduction in vigour score (p=0.03). A weak correlation was seen between a fall in Hb and POMS (p=0.09).

A higher pre-operative Hb did not reduce the required duration of in-patient physiotherapy (p=0.72).There was no correlation between

post-operative Hb and POMS (p=0.21) or required duration of in-patient physiotherapy (p=0.20).

A higher pre-operative grip strength predicted an early date of discharge by the physiotherapists (p=0.02).

Conclusion: We conclude that a fall in Hb of more than 4 g/dL has a detrimental effect on post-operative rehabilitation. Pre-operative grip strength measurements are valuable in predicting the rehabilitation potential of patients undergoing lower limb arthroplasty.

P96

THE ROLE OF PREOPERATIVE NEUROMUSCULAR ELECTRICAL STIMULATION IN TOTAL KNEE REPLACEMENT: A RANDOMISED CONTROLLED STUDY

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Objective: To investigate the possible effects of preoperative Neuromuscular Electrical Stimulation (NMES) to the quadriceps and hamstrings for the patients undergoing Total Knee Replacement (TKR) during the immediate postoperative period. Design: Prospective, randomised controlled study. Participants: 36 patients with osteoarthritis(OA) of the knee who were waiting for the elective TKR were randomly assigned to 1 of 2 groups (18 per group): control and study group. The study group patients received NMES to the quadriceps and hamstrings preoperatively. One patient in the control group and five patients in the study group were excluded due to the following reasons: operation postponed (two), unicompartmental knee replacement was performed (one), patients not willing to continue to use NMES due to personal reasons (two) and inappropriate/unable to use NMES (one). Both the control and the study group patients received standard physiotherapy postoperatively. Intervention: NMES (100Hz frequency; 0 to 55 volts amplitude; 1 second on/1 second off stimulation protocol) to the quadriceps and hamstrings muscle groups for 3 to 6 weeks preoperatively. Amplitude adjustments and usage timings were made by patients (at home) as dictated by the comfort level. Main outcome measures: Immediate postoperative assessment of straight leg raise, stair walking, flexion of knee, pain, walking distance, length of stay for rehabilitation and total length of hospital stay. Limitations: Small number of participants and only early followups were performed. Results: The outcome data suggest a possible benefit, but did not reach statistical significance in all but one parameter, early stair walking.

P97

CAN RECOVERY OF BONE MINERAL DENSITY AT THE FRACTURE SITE IN THE DISTAL RADIUS BE USED AS A MEASURE OF FRACTURE HEALING?

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Measurement of the rate of fracture healing is a major problem in fracture research. Bone mineral density (BMD) of fracture callus has been used as a measure of healing in diaphyseal fractures. However, metaphyseal fractures (especially in the elderly) are now the commonest type of fracture and are a significant public health problem. This study investigated whether measurement of BMD at the fracture site in the distal radius can be used as a measure of fracture healing.

We recruited 28 patients who had sustained a dorsally displaced distal radial fracture which was deemed to require treatment by intrafocal wire fixation. All patients had acceptable correction of dorsal and radial angle at final x ray (3 months). Wrist function was measured using the Patient Rated Wrist Evaluation (PRWE - a validated outcome measure for use after distal radial fractures), grip strength,and range of motion. All measurements were made at 6, 12 and 26 weeks. BMD was measured at the fracture site (examining the BMD of the medullary bone at the fracture site after removal of wires), in the opposite wrist and the lumbar spine using QCT at 6 weeks after fracture.

There was no correlation between fracture site BMD and BMD at the other wrist or the lumbar spine ($r < 0.3$). The BMD at the fracture site was higher than the BMD at the other wrist (mean 168 vs 70 HU; $p<0.001$ paired T test).There was no relationship between fracture site BMD or the ratio of BMDs fracture site / normal wrist, and any of the functional assessments (proportion grip strength recovered, range of motion or PRWE ($r < 0.3$)).

15 of these patients underwent a second QCT at 12 weeks after fracture. There was no significant change in fracture site BMD between the first and second scan.

These data indicate that fracture site BMD is unlikely to be a useful method of measuring metaphyseal bone healing. The increase in BMD at the fracture site was unexpected; possible explanations include impaction of bone or high BMD in woven bone (the relationship of which to bone stiffness is uncertain).

P98

OESTROGEN DEFICIENCY LEADS A DECREASE OF THE NUMBER OF CHONDROCYTES IN RABBIT GROWTH PLATE

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In the pubertal growth plate, sex hormones play important roles for the regulation of the proliferation, differentiation, maturation and programmed death of chondrocytes. Many studies have been reported on the regulation of oestrogen in long bone growth, however, some of the mechanisms have remained unclarified to date including its role for cell kinetics in the growth plate chondrocytes. The aim of this study was to clarify the effect of the deficiency of oestrogen on growth plate chondrocytes.

We obtained the growth plates of femoral head from the normal and ovariectomized Japanese white rabbits at 10, 15, 20 and 25 weeks. Ovariectomy was performed at 8 weeks. The cell kinetics of chondrocytes as defined by the numbers of proliferating and programmed dying cells was investigated using immunohistological methods.

The lengths of the femur were almost same both in the ovariectomised and normal rabbits. The height of the growth plate was larger in the former. The total number of chondrocytes in the ovariectomised rabbits was less than that of normal rabbits of the same age. Immunostaining of proliferating cell nucleous antigen (PCNA) showed a decrease number of proliferating chondrocytes and that of caspase-3 indicated a little increased number of apoptotic chondrocytes.

Oestrogen regulates endochondral bone formation through several pathways. It directly binds oestrogen receptor alpha and beta, and the former accelerates longitudinal bone growth whereas the latter represses it. Another pathway is through the GH-IGF-I axis: it closely interacts with GH and IGF-I for the control of longitudinal bone growth. In addition, there might be other mediators including transforming growth factor-beta, other IGFs and still unknown paracrine or autocrine factors as IHH PTHrP. Our study suggests that in the rabbit growth plate during puberty, oestrogen mainly acts through the GH-IGF-I axis since its deficiency declined the proliferating ability of chondrocytes, which led the decrease of the number of chondrocytes.

P99

WHAT IS IMPORTANT IN CARTILAGE REPAIR-MACROSCOPIC GRADING OR HISTOLOGY?

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Aims: To investigate

(1) the influence of histology on durability of cartilage repair following collagen-covered autologous chondrocyte implantation (ACI-C) in the knee.

(2) the relationship between macroscopic grading and durability of cartilage repair; and

(3) the relationship between macroscopic appearance and histology of repair tissue.

Patients and methods: The modified Cincinnati scores (MCRS) of eighty-six patients were evaluated prospectively at one year and at the latest follow-up (mean follow-up = 4.7yrs. Range = 4 to 7 years). Biopsies of their cartilage repair site were stained with Haematoxylin and Eosin and some with Safranin O and the neo-cartilage was graded as hyaline-like (n=32), mixed fibro-hyaline (n=19) and fibro-cartilagenous tissue (n=35). Macroscopic grading of the repair tissue using the international cartilage repair society grading system (ICRS)

was available for fifty-six patients in this study cohort. Statistical analyses were performed to investigate the significance of histology and ICRS grading on MCRS at 1 year and at the latest follow-up.

Results: The MCRS of all three histology groups were comparable at one year evaluation

(p=0.34). However, their clinical scores at the latest follow-up showed a significantly superior result for those with hyaline-like repair tissue when compared to those with mixed fibro-hyaline and fibro-cartilagenous repair (p=0.05).

There was no correlation between the ICRS grading and MCRS either at one year (p=0.12) or at the latest follow-up (p=0.16).Also, the ICRS grading of the repair tissue did not correlate with its histological type (p=0.12).

Conclusion: We conclude that any form of cartilage repair gives good clinical outcome at one year. At four years and beyond, hyaline-like repair tissue produces a more favourable clinical outcome. Macroscopic evaluation using the ICRS grading system does not reflect the clinical outcome or its durability or the histological type of repair tissue.

P100

TOPOGRAPHICAL GLYCOSAMINOGLYCAN VARIATION IN HUMAN ARTICULAR CARTILAGE

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Introduction: The load bearing status of articular cartilage has been shown to affect its biochemical composition. This study investigates the topographical variation of glycosaminoglycan (GAG) relative to DNA content in human distal femoral articular cartilage.

Methods: 26-paired specimens of distal femoral articular cartilage, from weight bearing and non-weight regions, were obtained from thirteen patients undergoing amputation. Following papain enzyme digestion, spectrophotometric (GAG) and fluorometric (DNA) assays assessed the biochemical composition of the explants. Data was analysed using a paired T test.

Results: Despite no significant differences in absolute DNA concentrations, weight-bearing regions of articular cartilage showed a significantly higher concentration of GAG relative to DNA compared with non-weight bearing areas (p=0.021).

Discussion: This study suggests that chondrocytes in weight bearing regions of human articular cartilage produce a greater quantity of GAG than those located in non-weight bearing areas. We conclude that mechanical loading is essential in maintaining the biochemical composition of human articular cartilage.

P101

CHONDROCYTE APOPTOSIS CORRELATES WITH ARTICULAR CARTILAGE DEGRADATION

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Apoptosis of articular chondrocytes may play an important role in the pathogenesis of osteoarthritis (OA). The aim of this study was to investigate the incidence of chondrocyte apoptosis in equine articular cartilage (AC) specimens and examine the relationship between the process of cell death and the degree of cartilage degradation.

The study comprised 2 populations of equine cartilage taken from the left forelimb. Population 1 (n=10) consisted of full depth cartilage from weight-bearing regions of equine metacarpophalangeal (MCP), proximal interphalangeal (PIP) and distal interphalangeal (DIP) joints. Population 2 (n=9) comprised cartilage from 6 different regions of the MCP joint: dorsomedial, dorsolateral, centromedial, centrolateral, palmaromedial and palmarolateral areas. Cartilage from each horse for each of the joints and joint regions was not always available. Seven micrometre cryostat sections were obtained. Haematoxylin and Eosin with Safranin-O stained sections were used to score structural differences between samples for features of cartilage pathology using a 'modified' Mankin scoring system. Two methods were used to quantify apoptotic chondrocytes: a direct method in which chondrocytes were assessed for morphological features of apoptosis using a light microscope and an immunohistochemical staining technique to detect the expression of active caspase-3 using a commercially available monoclonal antibody.

Apoptosis assessed by the direct method did not show any association with increasing severity of OA (r=0.11, p=0.7205). Overall there was a positive correlation between caspase-3 expression and cartilage damage (r= 0.44, p=0.0043). Caspase-3 expression was found to increase linearly with increasing severity of OA in the superficial, middle and deep zones of AC (r=0.36, p=0.0198; r=0.49, p=0.0011 and r=0.37, p=0.0237 respectively). Moreover, caspase-3 expression was higher in the superficial and middle zones than in the deep zone ($p<0.001$). In the superficial, middle and deep zones the expression of caspase-3 was higher in the MCP joint than the PIP joint ($p<0.05$, $p<0.01$ and $p<0.05$ respectively).

The significant positive correlation between disease severity and chondrocyte apoptosis, suggests that this process plays an important role in the pathogenesis of OA. The differences in the extent of apoptosis observed in different joints could be explained by the biomechanical environment of the joints.

P102

HISTOLOGICAL GRADING OF CARTILAGE IN ANTERO-MEDIAL OSTEOARTHRITIS OF THE KNEE

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Antero-medial osteoarthritis of the knee displays a well recognised pattern of cartilage damage on the medial tibial plateau. Anteriorly there is a full thickness cartilage defect, with transition to a partial thickness defect, becoming full thickness in the posterior third of the plateau. The retained posterior cartilage is macroscopically normal, but no previous study has assessed its histological features. This study characterises the histological changes, to examine if antero-medial OA of the knee represents a model of progressive osteoarthritic cartilage damage.

Five unicompartmental resection specimens of patients with idiopathic single compartment anteromedial osteoarthritis were assessed. The samples were stained with H&E and Saffinin-O stains and reviewed using the Mankin system, an established method for scoring osteoarthritic changes in cartilage (range 0 [normal] to 14 [grossly osteoarthritic]) Digital images of the histology were reviewed by two observers to exclude inter and intra observer error. Each specimen was assessed at 4 interval points (A,B,C,D) along the A-P axis starting from the most posterior aspect of the exposed bone to the area of macroscopically normal cartilage. Three repeat measurements were taken from the macroscopically normal region (D1,D2,D3). The scores were compared to historical age matched controls of non-osteoarthritic cartilage, where a Mankin grade of <3 suggests normal cartilage.

From anterior to posterior the H&E staining showed a consistent decrease in structural integrity and cellularity of the cartilage, matched by a qualitative decrease in GAG content (Saffinin-O staining). Mean Mankin scores showed a progressive decrease in score; A = 14.0 (95% CI 0), B = 5.8 (95%CI 2.4), C = 4.4 (95%CI 2.5), D = 1.0 (95%CI 0.9) {p=0.04 ANOVA}. Repeated measurements at the macroscopically normal area showed the Mankin grade was maintained; D1= 1.0 (95%CI 0.9), D2 = 0.6 (95%CI 0.5), D3 = 0.6 (95%CI 0.6).

The results show that the retained posterior cartilage in antero-medial arthritis has a consistently normal Mankin grade. We suggest the defect represents a model of progressive cartilage damage from near normal (posterior) to the grossly osteoarthritic state (anterior).

P103

ASSESMENT OF A NOVEL ANGIOGENIC FACTOR IN A SMALL ANIMAL MODEL OF ATROPHIC NONUNION

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Introduction: Atrophic nonunion is a well recognised complication of long bone fractures. Clinical trials show that BMP-2 accelerates healing and reduces nonunion in open tibial fractures. We are interested in a natural small molecule that has been previously demonstrated to stimulate angiogenesis in vivo. Our aim is to assess the two treatments in the prevention of nonunion. The small animal model we used is a non-critical size defect of the tibia deprived it of its blood supply by surgical stripping of the periosteum and curetting of the local endosteum thus closely reflecting the clinical situation.

The outcomes were measured by radiographic assessment and histology.

Methods: Wistar rats were treated with either the angiogenic molecule (0.1% or 0.003%), BMP-2 or vehicle alone (PBS) soaked in a type I collagen sponge. All animals underwent a 2mm osteotomy, stripping of the periosteum and endosteum proximally and distally for the length of the diameter of the tibia. Fluorescent markers were injected at 2 weekly intervals. The rats were sacrificed at 8 weeks. Both tibiae were disarticulated; fixator and soft tissues were removed and AP and lateral X-rays were taken. Subjective assessment of the healing on X-ray was carried out in two ways; using a radiographic scoring system and by grey scale analysis. The samples were embedded, sectioned and stained for new bone formation.

Results: Bridging or potential to bridge was seen in a number of animals on x-ray. Bridging or potential to bridge was judged to be present in 72.22% of the BMP-2 group and 66.67% of the high dose group compared to 22.22% of the control group. Histological analysis is being performed to confirm these findings.

Discussion: Atrophic nonunion is a serious clinical complication, unfortunately BMP-2 is a highly costly treatment option and therefore alternative molecular therapies are much sought after. We describe here an angiogenic molecule has some potential in preventing formation of nonunion.

P104

SUSCEPTIBILITY OF INTERVERTEBRAL DISC CELLS AND OTHER CELL TYPES TO APOPTOSIS

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Cells of the intervertebral disc exist in an unusual environment compared to those of other tissues. Within the disc there are low levels of nutrients available, low oxygen levels and it is an acidic environment due to high lactate levels. Apoptosis (programmed or controlled cell death) has been reported in intervertebral discs, as well as necrosis (uncontrolled cell death). This study has focused on examining the sensitivity of nucleus pulposus (NP) cells to several stimuli, in comparison to two other cells types.

Ultra violet (UV) irradiation, serum starvation (with no foetal calf serum) and treatment with 2mM hydrogen peroxide were used to induce apoptosis in cultured bovine NP cells, HeLa (cancer cell line) and 293T cells (human embryo kidney derived) cells. Apoptosis was identified by nuclear morphology following staining with fluorescent Hoechst 33342 dye and propidium iodide; the incidence was measured at 24, 48 and 72 hours. Untreated controls were used for each treatment and at each time point.

The incidence of apoptosis increased with time for all treatments. After 72 hours, UV treatment produced the highest levels of apoptosis with levels of apoptosis occurring in the order of HeLa (94%) > NP cells (29%) > 293T cells (15%). Treatment with hydrogen peroxide and serum starvation induced apoptosis at lower levels in all three cell types (maximum of 30%). Serum starvation induced apoptosis in only 10% of NP cells at 72 hours, compared to 20% in HeLa cells. None of the controls contained apoptotic cells.

NP cells are stimulated to apoptose in response to UV irradiation, hydrogen peroxide and serum starvation. However, levels of apoptosis are much lower after UV treatment in comparison to HeLa cells (3 times lower), suggesting that they may have a protective mechanism to this apoptotic stimulus, compared to HeLa cells. The low levels of apoptosis observed in NP cells with serum starvation may be due to the low nutrient environment that they exist in normally.

P105

ESTABLISHMENT OF AN EXTERNALLY FIXATED RAT FEMORAL FRACTURE MODEL

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Background & Objectives: The objective of this study was to develop a rat model of fracture repair. Fixation of experimental fractures is generally internal {Kirschner wire/intramedullary (IM) nail} or external (single/double plane devices). Internal fixation using the IM-fixated model of a standard closed fracture is well described in rats. However, nail insertion can disrupt fracture site morphology and limit x-ray analysis. We planned to create an externally fixated femoral model, to optimise our outcome measures and facilitate the further investigation of bone healing within the department.

Methods: A simple four pin unilateral external fixator was designed and constructed from four stainless steel pins, secured to a stainless steel plate with nuts. Forty-one female Sprague-Dawley rats, (12-18wks), were used. Following anaesthesia the right femur was exposed and a mid-femoral osteotomy made prior to fixator application. Post-operative x-rays were taken to confirm reduction. Animals were assigned to groups for biomechanical strength testing (BST) or histology. Fifteen animals (fractured and contralateral limbs) were sacrificed at 4, 6 or 8 weeks for BST (four-point bending). Maximum load to failure was recorded and stiffness calculated from the load displacement curve obtained. Both parameters were standardised as a percentage of the contralateral limb. Twenty-five fractured limbs were used for histological analysis at day 4, and 1, 2, 4, 6 or 8 weeks.

Results: Satisfactory reduction was confirmed in all animals post operatively and no complications were noted. Histological assessment at day 4 demonstrated a predominantly lymphocytic inflammatory response within the fracture haematoma. This was replaced with endosteal and periosteal new bone between weeks 1 and 2. Bridging of the fracture gap was seen at week 6. Stiffness and load to failure increased with increasing time. There was a statistically significant improvement in the percentage stiffness ($p=0.035$) and load to failure ($p=0.012$) between 4 and 8 weeks.

Conclusion: A simple reproducible externally fixated rat model has been established and characterised by radiography, histology and four point bending. This model has since proven to be of value in the study of the role of lipid lowering and anti-inflammatory drugs as well as cell therapy on fracture repair.

P106

INFLAMMATORY CHEMOKINE EXPRESSION IN PRIMARY HUMAN OSTEOBLASTS INDUCED BY COBALT PARTICLES

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Wear debris is a key factor in the pathophysiology of aseptic loosening of orthopaedic endoprostheses. Cobalt-chromium-molybdenum (Co-CrMo) alloys are used for metal-metal hip implants due to their enhanced wear resistance profiles. Whilst these alloys have widespread clinical application, little is known about their direct effect on osteoblast biology. To address this issue, in this study we have investigated particle-mediated inflammation, as a putative mechanism of aseptic loosening. The effects of Co2+ ions on the bone cellular milieu were assessed in vitro by profiling of classical inflammatory mediators. The inflammatory driver PGE2 was quantified and found to be increased, following osteoblast stimulation with metal ions, suggesting the initiation of a local inflammatory response to metal particle exposure. To determine the biological import of this molecular event, the role of metal ions in recruiting inflammatory cells by chemokine production was assessed. These data demonstrated significant induction of the chemokines, IL-8 and MCP-1 following both 12 and 24 hour exposure to 10ppm of Co2+. In this study, we demonstrate that Co2+ particles can rapidly induce chemotactic cytokines, IL-8 and MCP-1 early stress-responsive chemokines that function in activation and chemotaxis of monocytes, and PGE2, which stimulates bone resorption. We have shown that this induction occurs at a transcriptional level with significantly increased mRNA levels. These data lend further weight to the hypothesis that wear mediated osteolysis, is due, at least in part, to underlying chronic inflammation.

P107

THE INFLUENCE OF EARLY NUTRITIONAL COMPROMISE ON BONE STRUCTURE AND STRENGTH

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Introduction: Epidemiology suggests that an intrauterine nutrient restriction increases the likelihood of osteoporosis in later life, possibly due to differences in bone structure and strength. We hypothesise that, in an ovine model, early nutritional compromise reduces vertebral cancellous bone density and cortical thickness, and thereby reduces vertebral compressive strength.

Materials and methods: Lumbar spines were dissected from 8 sheep (6 male, 2 female: mean age 2.7 yrs). Spines were divided into different groups, based on the early diet of the sheep: group CC received a control diet, group IU received low protein in utero, and group PN received low protein both in utero and postnatally. Fifteen motion segments (consisting of two vertebrae and the intervening disc and ligaments) were prepared from the spines, and compressed to failure using a hydraulically-controlled materials testing machine to obtain yield strength. 1mm-thick bone slices were taken from the mid-sagittal and para-sagittal regions of each vertebral body and micro-radiographed. Digital images of the micro-radiographs were analysed to obtain the cancellous bone density in anterior and posterior regions, and the cortical thickness in the anterior, posterior, superior and inferior regions. Repeated measures ANOVA was used to test for differences in parameters at the different locations, and between the groups.

Results: The anterior cortex was 28% thinner for the IU group, and 23% thinner for the PN group compared to controls (both $p<0.001$). In the PN group, the superior cortex was also 18% thinner than controls ($p<0.02$). There was no significant difference between cancellous bone density in either region. Yield strength was 16% lower in the IU group compared to controls, but this did not reach significance.

Discussion: In the nutritionally compromised groups, cortical thickness was lower in regions of the vertebral body where fractures often occur in elderly people. However, the reduction in cortical thickness is not accompanied by a significant reduction in compressive strength in the sheep model. These findings suggest that the well-maintained cancellous bone protects the vertebra from fracture.

P108

TGF-BATE1 DOSE AND CELLULAR DENSITY-DEPENDENT EFFECT ON CHONDROGENIC DIFFERENTIATION OF HUMAN BONE MARROW STEM CELLS

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The repair of cartilage defects remains a significant clinical challenge.The use of mesenchymal stem cells for cell-based tissue-engineering strategies represents a promising alternative for the repair of the defects. In this study, we investigated the TGF-bate1 dose and cellular density-dependent effect on chondrogenic differentation of human bone marrow-derived mesenchymal stem cells (MSCs) cultured in alginate beads in vitro.

Methods A volume of 6 ml bone marrow was collected from six volunteer donors respectively. MSCs were cultured in different cellular density (1_104, 1_105, 1_106 and 5_106/ml) and treated with different doses of TGF-beta1 (0, 1, 10, 50 and 100 ng/ml). Immunohistochemistry and in situ hybridization were applied to detect the expression of collagen type II and assay proteoglycan in different time internal.

Results 95% cellss were alive after density gradient centrifugation. BMSCs had a similar spindle-like morphology. Type II collagen and proteoglycan were showed positive staining in the 10 ng/ml TGF-beta1 group, weakly positive in the 50 ng/ml and 100 ng/ml group, negative in the 0 ng/ml and 1 ng/ml group. With time, the proteoglycan quantity increased. All cell density groups except 1_104/ml showed positive expression of collagen type II and proteoglycan synthesis, and better staining with increase of cellular density. Proteoglycan synthesis did not increased until the fifth weeks.

Conclusions The chondrogenesis differentiation of human MSCs is dose-dependent. 10ng/ml TGF-beta1 is a suitable concentration for such inducing. The cellular density is also important for the differentiation of MSCs. Too small density is ineffective. The more cells, the better differentiation. And the time of in vitro culture should not be longer than 4 weeks

P109

THE EFFECTS OF PHORBOL MYRISTATE ACETATE (PMA) AND N-FORMYL-MET-LEU-PHE (FMLP) ON LEUCOCYTE ADHESION AND ACTIVATION

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Leucocytes are white blood cells that help the body fight against bacteria, viruses and tumour cells. However, the activity of leucocytes has been implicated in other clinically important inflammatory conditions such as ischaemic heart disease, stroke, and during cardio-aortic and orthopaedic surgery.

The main objectives of this study was to optimise methods for the isolation of leucocyte subpopulations (neutrophils and monocytes), and to assess in vitro the effects of PMA and fMLP on markers of leucocyte adhesion (CD11b, CD62L) and activation (intracellular hydrogen peroxide) (n=10). Leucocyte subpopulations were labelled by incubation with fluorescein isothiocyanate (FITC) conjugated anti-human CD11b and CD62L antibodies. The cell surface expression of these labelled adhesion molecules were measured by flow cytometry. Intracellular production of hydrogen peroxide by neutrophils and monocytes was measured by flow cytometry, using the fluorochrome dichlorofluorescin diacetate (DCFH-DA). These were visualised by Immunofluorescence microscopy.

During this study, methods of isolating leucocyte subpopulations from whole blood were optimised. This ensured that these cells were isolated with consistently high yields, purity and with no changes in cellular function. Following incubation with PMA and fMLP, neutrophils and monocytes displayed an increase in CD11b cell surface expression; a decrease in CD62L cell surface expression; and increased leucocyte activation. Leucocyte activation was represented by the intracellular production of hydrogen peroxide.

In conclusion this study confirms that both PMA and fMLP have an intrinsic effect on markers of leucocyte function. These findings are in agreement with previous studies performed.

Key words: Leucocytes, Inflammation, PMA (Phorbol Myristate Acetate), fMLP (N-Formyl-Met-Leu-Phe)

P110

AUTOLOGOUS CHONDROCYTE IMPLANTATION VERSUS MATRIX-INDUCED AUTOLOGOUS CHONDROCYTE IMPLANTATION FOR OSTEOCHONDRAL DEFECTS OF THE KNEE: MINIMUM 2 YEAR FOLLOW-UP RESULTS

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Purpose: We report on minimum 2 year follow-up results of 71 patients randomised to autologous chondrocyte implantation (ACI) using porcine-derived collagen membrane as a cover (ACI-C) and matrix-induced autologous chondrocyte implantation (MACI) for the treatment of osteochondral defects of the knee.

Introduction: ACI is used widely as a treatment for symptomatic chondral and osteochondral defects of the knee. Variations of the original periosteum-cover technique include the use of porcine-derived type I/type III collagen as a cover (ACI-C) and matrix-induced autologous chondrocyte implantation (MACI) using a collagen bilayer seeded with chondrocytes.

Results: 71 patients with a mean age of 33 years (15-48) were randomised to undergo either an ACI-C or a MACI. 37 had ACI-C and 34 MACI. The mean size of the defect was 5.0cm2. Mean duration of symptoms was 104.4 months (9-456). Mean follow-up was 33.5 months (24-45). Functional assessment using the modified Cincinnati knee score, the Bentley functional rating score and the visual analogue score was carried out. Assessment using the modified Cincinnati knee score showed a good to excellent result in

57.1% of patients followed up at 2 years, and 65.2% at 3 years in the ACI-C group; and 63.6% of patients at 2 years, and 64% at 3 years in the MACI group. Arthroscopic assessments showed a good to excellent International Cartilage Repair Society score in 81.8% of ACI-C grafts (22 patients) and 50% of MACI grafts (6 patients). Fisher's exact test showed a p value of p=0.35 (not statistically significant). Hyaline-like cartilage or hyaline-like cartilage with fibrocartilage was found in biopsies of 56.3% of the ACI-C grafts (9 out of 16 patients) and 30% of the MACI grafts (3 out of 10 patients) after 2 years. Fisher's exact test showed a p value of p=0.25 (not statistically significant).

Conclusion: At this stage of the trial we conclude that the clinical, arthroscopic and histological outcomes are comparable for both ACI-C and MACI.

P111
TO ASSESS THE CHONDROGENIC POTENTIAL OF HUMAN EMBRYONIC STEM CELLS IN 3D CULTURE

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Aim: To investigate the directed chondrogenic differentiation of human embryonic and adult stem cells in 3D alginate bead culture.

Introduction: Cartilage possesses limited self-renewal potential and current repair of damage due to trauma or disease involves removal of non-load bearing chondrocytes from a healthy part of the joint, expansion of chondrocytes and subsequent surgery to replace damaged, load-bearing cartilage. We investigated the potential of human embryonic and adult stem cells as an alternative cell source for cartilage repair.

Experimental design: Human embryonic stem cells (hESC) and human adult marrow stromal cells (hMSCs) cells were cultured in alginate in a 3D bead format in control or chondrogenic media over a 21day period. Cells were subsequently released from their matrix for gene expression analysis or fixed within alginate beads and crytostat sections prepared for immunostaining and histology.

Cell types used: H9 human embryonic stem cells, bone-marrow derived hMSCs and HEK293 (human embryonic kidney epithelium cell line, used as a negative control).

Data: H9 and hMSC cells cultured in alginate beads bathed in control media have a denser matrix with no lacunae-like structures compared to those cultured in the presence of chondrogenic media. The presence of chondrogenic media results in a matrix containing cells within lacunae-like structures very similar to those seen in human cartilage. In contrast, HEK293 cells formed large highly cellular clusters which had clearly undergone significant proliferation. As both H9 and HEK293 cells are highly proliferative the reduction in the proliferative potential of the chondrogenic H9 derived cells is consistent with entry into a stable terminally differentiated state.

Immunostaining demonstrated that hMSCs and H9 cells express cartilage specific Collagen II and Collagen X.

Conclusion: 3D culture of adult hMSCs and hESC (H9) in alginate beads has resulted in stable directed differentiation down the chondrogenic lineage. These data point towards the future use of these human cell sources in cartilage repair.

P112
THE OSTEOGENIC POTENTIAL OF BONE SUBSTITUTE MATERIALS SUBJECTED TO PHYSIOLOGICAL STRAINS IN VITRO

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Bone substitutes have emerged as a promising alternative in surgeries requiring bone grafting, with a large array of materials available for today's surgeon. Unfortunately, there is currently no definitive method for comparing the potential bone-healing potential of these different materials. We have developed a novel technique for assessing the osteogenic capacity of different bone substitutes in a mechanically-stimulating perfusion bioreactor.

The Zetos(TM) bioreactor system consists of individual flow chambers connected to a low-flow perfusion pump, which recirculates media through samples. The Zetos can be programmed to apply a controlled stress or a controlled strain to each individual sample inside the flow chamber. Since bone formation has been shown to be optimal with short doses of high amplitude strains, test samples were subjected to daily loading corresponding to physiological strain experienced during a jumping exercise (maximum 3000 microStrain).

Three substitute materials representing the range of materials available clinically were tested in the Zetos system; these included collagen, calcium phosphate, and a synthetic polymer. Primary human osteoblasts were seeded onto the substitutes, which were then placed inside the Zetos system and maintained under load or non-load conditions for 14 days. No supplementary osteogenic factors were provided to the cells. The degree of bone formation in the samples was assessed using Von Kossa staining and quantified in terms of the area of new mineral relative to the surface area of the substitute.

No mineralisation was detected in the non-loaded samples. However, in the loaded samples, mineralisation was detected in some of the substitutes. The degree of mineralisation depended on the material: in collagen, an average of 0.22 mm2/mm2 was mineralised; in calcium phosphate, mineralisation averaged 0.0013 mm2/mm2; but in the loaded polymer samples, no mineralisation was detected.

This indicates that mechanical loading is a sufficient stimulus for bone formation in some materials, even in the absence of other known osteogenic factors. Further, commercial substitutes differ in their ability to support bone formation under conditions of physiological loading. Further development of this technique could allow it to be used as a screening tool for predicting the efficacy of commercial products.

P113
THE EFFECT OF CYTOMODULIN-1 ON THE PROLIFERATION AND DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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A combination of stem cell therapy and tissue engineering is emerging as one of the most promising approaches for skeletal tissue repair and regeneration. Osteoinduction of human bone marrow mesenchymal stem cells (MSCs) is initiated through local signals or growth factors, of which the bone morphogenetic proteins (BMPs) are the best characterised. Cytomodulin-1 (CM-1), a synthetic heptapeptide with functional similarity to members of the TGF-B super family, has been classified as a novel growth factor associated with osteoinduction of MSCs. However, the effects of CM-1 on human bone MSCs are still unclear. The aim of this study was to determine any effects for CM-1 and its scrambled control (CM-1 SCRAM) on the proliferation and differentiation of human bone marrow MSCs along the osteogenic lineage.

Primary human bone marrow MSCs were cultured in the presence of CM-1 and CM-1 SCRAM at a range of concentrations (10-8M - 10-6M) in vitro for up to three weeks. 100 ng/mL of recombinant human BMP-2 (rhBMP-2) was used as a positive control. At the end of the culture period, histological and biochemical assays were carried out on the cultures.

Biochemical assays revealed that 10-7M of CM-1 significantly stimulated alkaline phosphatase specific activity compared with the negative control group ($P<0.05$) in a similar way to the rhBMP-2 positive control group. These data were supported by an observed increase in positive alkaline phosphatase staining in the 10-7M of CM-1 and rhBMP-2 treated cells. However, total DNA content was not significantly different between any of the groups.

This study indicated the potential of using CM-1 as an osteogenic growth factor for skeletal tissue regeneration which may provide an alternative approach to meet the major clinical need in orthopaedics and craniofacial surgery.

* Cytomodulin-1 and the scrambled control were genuine gifts from Professor (emeritus) Rajendra S. Bhatnagar at the Department of Bioengineering, University California Berkley, USA.

P114

JUNCTIONAL HISTOLOGY OF BIOLOGIC FIXATION AS GRAFT FIXATION METHOD IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTIVE SURGERY: CLINICAL CORRELATION OF EXPERIMENTAL STUDIES

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To illustrate our clinical experience of using a complete biological method of fixation in ACL surgery and correlate the histology at the graft and the host bone interface performed in an animal experiment.

Materials: 18 male patients of mean age were 31.2 years (range 18 to 50 years) were operated on. The autogenous graft prepared from lateral part of the quadriceps aponeurosis, part of the patella and ligament leaving distal tibial attachment, passed through the trans-osseous tunnel so that bony part of the graft stay within the femoral tunnel, remaining part was sutured with the ilio-tibial tract.

Patients began immediate knee exercises with continous-passive-motion devices in the recovery room. With 100 degrees of knee motion, they allowed to bear full weight on the operatively treated limb with knee in a brace in extension

Results: 3 patients had superficial wound infection and 2 had haemarthrosis. None had any laxity or flexion contracture, mean flexion arc was135 (130-145) degree.

Conclusion: Histology of the bone graft and host tunnel confirms full incorporation of the graft in experimental animals performed by our senior author. The procedure of biologic fixation method in ACL reconstruction surgery to preserve the biological integrity of the patellar ligament distally in the tibial end may avoid early failure in fixation method. The biological integration producing a bone block in the femoral tunnel may enable clinician to start early rehabilitation program.

P1

MOLECULAR MODELING COMPARISON OF NITROGEN-CONTAINING BISPHOSPHONATES OF VARYING POTENCY CO-CRYSTALIZED IN FARNESYL DIPHOSPHATE SYNTHASE

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Farnesyl diphosphate synthase (FDPS) is a metabolic enzyme that has been demonstrated to be a major molecular target of nitrogen-containing bisphosphonates (N-BPs). It has been known for many years that minor changes to the structure of N-BPs can dramatically affect their antiresorptive potency. However, a detailed explanation has been lacking. Progress on the generation of co-crystal structures of this enzyme in the presence of several potent and weak N-BP inhibitors has provided an opportunity to examine the precise way in which bisphosphonates act on FDPS. The active site of FPPS contains two substrate binding sites (for GPP/DMAPP and for IPP). The current protein crystallographic evidence shows that risedronate and zoledronate occupy the GPP/DMAPP site. We have therefore begun to investigate whether wide ranging inhibitory potency differences observed among N-BPs in vivo and as inhibitors of the human enzyme can be explained by key interactions within the GPP/DMAPP site.

We have now acquired additional co-crystal data on several potent and weakly active N-BPs. These structures all closely compare with the PCP binding characteristics of risedronate. Also, key interactions of the critical nitrogen residue with Threonine 201 -OH and the Lysine 200 backbone C=O were observed with all potent N-BPs exhibiting appropriate NHO angle of greater than 125° and a N-O distance of 3.1Å or less. However, the weakly active N-BPs, NE-11809 and NE-58051, display longer N-O distances of 7.01Å and 4.58Å respectively, explaining their lack of activity. Furthermore, we have successfully predicted these binding modes utilizing computational modeling techniques. Additional active analogs were also modeled. Thus, NE-11808, NE-11807, and NE-97220 exhibited similar H-bonding characteristics involving the nitrogen of the N-BP. Comparative analyses of structurally and clinically relevant N-BP's is now possible with these techniques. With the correlations observed, the GPP site and the accessibility of these hydrogen bonding sites to the nitrogen moiety is further established as the key explanation for the differences in potency of N-BPs.

P2

EARLY LIFE - CALCIUM SENSING RECEPTOR GENE POLYMORPHISM INTERACTION IN DETERMINATION OF ADULT BONE MASS: THE HERTFORDSHIRE COHORT STUDY

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Several studies have looked for evidence of an association between one single nucleotide polymorphism (SNP) in the calcium sensing receptor gene (CaSR) with bone mineral density (BMD), but results have been largely negative. We have previously found evidence of interaction between growth in early life and SNPs of the vitamin D receptor and growth hormone genes in the determination of BMD. Here, we looked for evidence of interaction between growth in early life and SNPs of the CaSR gene in the Hertfordshire Cohort Study.

Four hundred and ninety eight men and 468 women aged 59-71 years were recruited. Birth-weight and weight at one year of age were available from historical ledgers. A lifestyle questionnaire was administered and BMD at the lumbar spine and femoral neck measured. DNA was obtained from whole blood samples using standard extraction techniques. Five SNPs in the CaSR gene termed CASRV001 (rs1801725, G>T, S986A), CASRV002 (rs7614486, T>G, untranslated), CASRV003 (rs4300957, untranslated), CASRV004 (rs3804592 G>A, intron), and CASRV005 (rs1393189, T>C, intron) were analysed.

We found no significant associations between BMD at the lumbar spine or total femur with any of the SNPs studied. However, among women the 11 genotype of the CASRV003 SNP was associated with higher lumbar spine BMD within the lowest birth-weight tertile, while the opposite pattern was observed among individuals in the highest tertile of birth-weight (test for interaction on 1df p=0.005, adjusted for age, BMI, physical activity, dietary calcium intake, cigarette and alcohol consumption, social class, menopausal status and HRT use). Similar relationships were seen at the total femur (p=0.042, fully adjusted). In addition, the 1 allele of the CASRV3 SNP was associated with higher total femoral BMD among women in the lowest weight at one-year tertile, while the opposite pattern was again observed among individuals from the highest tertile of weight at one year (p<0.001 fully adjusted).

In conclusion, we have found evidence of an interaction between one SNP in the CaSR gene and growth in early life in the determination of BMD in a UK female population in late middle age.

P3

EVIDENCE FOR INTRINSIC SNARE-DEPENDENT GLUTAMATE RELEASE AND ITS ROLE IN MESENCHYMAL STEM CELL FATE ALLOCATION AND CELL SURVIVAL

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Skeletal development, repair and remodelling are dependent on the proliferation and differentiation capacity of mesenchymal stem cells (MSCs), which reside in the bone marrow and give rise to different mesenchymal lineages. The regulatory inputs that control MSC fate are unclear. In this study we have identified a fundamental role for glutamate signalling in the endogenous regulation of MSC proliferation and fate determination and provide evidence for autocrine glutamatergic signalling mediated by glutamate released locally by MSCs into their extracellular microenvironment. Using a combination of RT-PCR, fluorescent immunolocalisation and confocal calcium imaging we have identified expression and function of ionotropic and metabotropic glutamate receptors by human MSCs (hMSCs). In MTT and CFU-f assays glutamate receptor activation increased MSC viable cell numbers while antagonists impaired proliferation, significantly reduced colony formation without affecting apoptosis and prevented their osteogenic differentiation determined by alkaline phosphatase activity, von-Kossa staining and Anexin V/PI flow cytometry. Using a fluorimetric assay of glutamate exocytosis we demonstrated that hMSCs exhibit spontaneous calcium-dependent glutamate release at levels comparable to neurones and in a manner dependent on their osteogenic differentiation status. By FM1-43 and acridine orange labelling we provide evidence that hMSCs contain vesicles that actively undergo cycles of fusion, exocytosis and recycling analogous to those observed at glutamatergic synapses in the CNS. By molecular profiling we have identified the expression of all of the fundamental molecular machinery required for calcium-dependent glutamate exocytosis including the vesicular v-SNARE VAMP and the plasma membrane target t-SNARE syntaxin, whose interactions facilitate local and directed vesicular neurotransmitter release at central glutamatergic synapses. Additionally, we demonstrated that MSCs express the vesicular glutamate transporter vGlut1, whose expression is both necessary and sufficient to induce a glutamatergic phenotype on neurones and activity is required for packaging glutamate into synaptic vesicles prior to exocytosis. Taken together these data strongly suggest that endogenous autocrine glutamate signalling is required to maintain MSC viability and regulate differentiation. The manipulation of this pathway may provide a novel mechanism of regulating the activity of MSCs in bone disorders such as osteoporosis and cell and tissue engineering approaches to disease therapy.

P4

APOPTOTIC OSTEOCYTES INDUCE THE PRODUCTION OF OSTEOCLASTOGENIC CYTOKINES

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Apoptotic bodies elicit in phagocytic cells behavioural responses that have far reaching effects on tissue function. Recent evidence points to the production of signals from the dying osteocyte that might target the activity of bone resorbing cells either directly or indirectly via signals generated in the bone resident osteoblast population. In this study we introduced osteocyte apoptotic bodies (OAB) to osteoblast cultures in order to test whether they can engender the production of

secondary signals capable of modifying osteoclast precursor behaviour.

Apoptosis was induced in osteocyte cultures in response to serum deprivation and apoptotic bodies were purified by Annexin-V-FITC binding and paramagnetic bead-conjugates. OAB were then presented to osteoblasts in culture for 24 hours. Supernatants were centrifuged to remove cell debris and incubated with an array of cytokine-specific capture beads and detection antibodies that enabled the simultaneous analysis of multiple cytokines.

We observed an increase in a number of cytokines that are known to induce osteoclast formation and activation as well as monocyte differentiation and recruitment to sites of bone remodelling and injury. In particular, we observed a 9-fold increase in TNF-a production, 14-fold increase in IL-6 production, 2-fold increase in IL-1b production, 4-fold increase in MCP-1 production and 20-fold increase in GM-CSF production in osteoblast cultures in response to OAB. Following on from these findings we observed that incubation of osteoclast precursor monocyte cultures with supernatants collected from osteoblast cultures that had been fed with apoptotic osteocytes, induced osteoclast formation and activation.

This study has demonstrated that apoptotic products released by osteocytes induce the subsequent production by osteoblasts of an array of cytokines involved in osteoclast and monocyte recruitment and activation. In this way, the local induction of osteocyte apoptosis might be targeting the resorption process to particular sites in bone. While the identity of the apoptotic osteocyte-resident molecules responsible for these effects is still unknown a further understanding of this signalling mechanism might lead to novel intervention strategies in a number of bone diseases.

P5

VALUE OF VERTEBRAL FRACTURE ASSESSMENT IN IDENTIFYING VERTEBRAL FRACTURE AT DXA: A CASE-CONTROL STUDY

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Vertebral fracture assessment (VFA) has been hailed as a new tool to identify vertebral fracture at the time of DXA. Studies comparing results with xrays show a high negative predictive value, i.e ability to exclude fracture. New systems use computer aided placement of morphometry points, making it feasible to offer VFA as part of a clinical densitometry service. To be an effective tool VFA must enable clinicians to distinguish osteoporotic fractures from degenerative or other deformity.

This study compared VFA results in a peripheral fracture cohort at high risk of osteoporosis and potentially vertebral fracture, with controls. Fracture Cases (FC) were identified by our fracture liaison service with incident low impact fractures and aged 65 and over. Community controls [C] from a GP practice had no fractures or known osteoporosis risk factors. All completed osteoporosis risk questionnaires and had bone densitometry on a Hologic 4500c DXA Bone Densitometer upgraded with 'Discovery' software to perform 'Instant vertebral assessment' with CADfx. Vertebral deformities were graded automatically using Genant semi-quantitative technique.

217 FCs were assessed (191F: 26M, mean age(SD) 73yrs(4.32) and 60 controls (42F:18M, mean age(sd) 71yrs(4.51). More FCs were osteoporotic (FC 44.7%, C 6%, OR 11.3, p<0.001) and kyphotic (FC 23%,C2%,OR 17.6, p<0.001). There was no difference in the prevalence of back pain. 63% FCs had some spine deformity on VFA, C 43%, OR2.2, p<0.01. However, mild and moderate deformities were equally prevalent in FCs and controls. Only severe deformities were more prevalent in FCs (13%; C 1.6%, OR 8.7, p=0.01). Osteoporotic FCs had more deformities (65/97) than FCs with normal or osteopenic BMD (39/120) (OR4.2, p<0.0001)

The high prevalence of vertebral deformity in FCs, especially where osteoporotic, makes it likely that VFA is identifying new undiagnosed vertebral fractures. However the prevalence of deformity in elderly controls means VFA should not be used in isolation in clinical service to screen for vertebral fracture, but it provides a novel screening tool to indicate a need for spine xray. VFA may be important to help identify patients with vertebral fracture warranting potent osteoporotic treatment and possibly PTH injections.

P6

REGENERATION POTENTIAL OF OSTEOPROGENITOR CELLS DERIVED FROM HUMAN EMBRYONIC AND ADULT STEM CELLS IMPLANTED IN A RAT CALVARIAL LESION

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Embryonic stem cells provide an excellent renewable source of osteogenic cells for use in bone repair. While osteoprogenitor cells can be derived from human embryonic stem cells (hES) and adult bone marrow stromal cells (hMSC) in vitro their potential to repair bone in vivo remains unclear.

Aim: to compare the ability of hES and hMSC derived osteoprogenitor cells to promote repair during the first week after in vivo implantation in a calvarial defect in both immuno-competent and immuno-deficient rat strains.

Methods: HMSC cells were isolated from femoral bone samples harvested from patients undergoing orthopaedic surgery. The hES cells line H9 cells were expanded and derived from embryoid body aggregates. hMSC and hES cells were pre-treated with osteogenic factors for 4 days and mixed with human demineralised bone matrix (DBM: Allosource®) prior to in vivo implantation. HES and hMSC derived cells in DBM or DBM alone (control group) was implanted into an experimentally created full-thickness calvarial defect in adult immuno-competent Sprague-Dawley male rats (n=3) and in immuno-deficient (RNU-Foxn^{rnu}) male rats (n=3). Tissues were collected 7 days after surgery, cryosectioned and representative sections were stained to quantify the area of new bone formation. Implanted human cells were identified with fluorescence in situ hybridization for human DNA.

Results: Human ES and MSC cells were detected within the defect area in both strains of rats. Newly formed bone was evidenced close to the edges of the lesion and associated with DBM particles in all the experimental groups. When implanted into immune-competent animals hMSC or hESC derived osteoprogenitor cells did not engender more new bone formation than control (DBM) groups. In contrast, in immune-deficient animals implantation of both hMSC and hESC derived cells increased significantly the amount of bone formed in the lesions relative to controls (DBM). HMSC and hESC derived cells engendered 3 times and 10 times the amount of new bone formation measured in controls (DBM) respectively (P<0.001).

Conclusion: These data point to the potential efficacy of adult and embryonic stem cell derived osteoprogenitors and to the rapid bone forming potential of hESC derived cells that have undergone directed osteogenic differentiation in vitro.

P7

BONE MINERALISATION IN VITRO IS INHIBITED BY EXTRACELLULAR NUCELOTIDES

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Extracellular nucleotides, signalling through P2 receptors, may play an important role in bone biology, modulating both osteoblast and osteoclast function. Osteoblasts express multiple P2 receptor subtypes, including $P2X^2$, $P2X^5$, $P2X^7$, $P2Y^1$, $P2Y^2$, $P2Y^4$ and $P2Y^6$ receptors. Primary osteoblast cultures were obtained from neonatal rat calvariae by trypsin/collagenase digestion and cultured for up to 21 days in the presence of nucleotide agonists (1-100 micromolar ATP, ADP, UTP, UDP, CTP, BzATP); bone nodule formation was assessed by image analysis of cell layers stained with alizarin red to demonstrate calcium deposition. ATP, UTP, CTP and Bz-ATP (> 1 micromolar) inhibited bone nodule formation by up to 95%, whereas ADP and UDP were without effect. Light microscopy revealed that although ATP and UTP-treated osteoblasts deposited abundant fibrous collagenous material with the characteristic morphology of bone nodules, mineralisation failed to occur. Moreover, in ATP or UTP-treated (10 micromolar) cultures, alkaline phosphatase (ALP) activity was reduced by up to 65%. ATP and UTP, but not ADP and UDP, decreased the expression of ALP and osteocalcin mRNA in rat osteoblasts. The potent inhibitory actions of ATP and UTP are

consistent pharmacologically with mediation by the $P2Y^2$ or $P2Y^4$ receptor subtypes. Reactive blue 2, a $P2Y^4$ receptor antagonist, failed to prevent the nucleotide-induced block of mineralisation, suggesting that $P2Y^2$ receptor activation may lead to the functional effects of ATP and UTP. In agreement, $P2Y^2$ receptor knockout mice displayed increased bone mineral content in the femora (9%) and tibia/fibula (17%) compared to wildtype mice. Furthermore, as shown by RT-PCR, western blotting and immunofluorescence, expression of the $P2Y^2$ receptor was increased strongly in mature, bone-forming osteoblasts. However, several ecto-nucleotidases can generate pyrophosphate (PPi) from nucleotide triphosphates; since PPi is a potent inhibitor of bone mineralisation (at concentrations > 1 micromolar in our in vitro system), the possibility remains that the effects of extracellular nucleotides could also occur independently of P2 receptors. Thus, the data presented here suggest that purines and pyrimidines could function as local signalling agents that 'switch off' bone mineralisation, acting through the $P2Y^2$ receptor, or possibly also via a direct inhibitory effect of PPi on hydroxyapatite crystal formation.

P8

DISTINCT EFFECTS OF OESTROGEN AT PERIOSTEAL AND CANCELLOUS BONE ENVELOPES MAY REFLECT DIFFERING REQUIREMENTS FOR ANGIOGENESIS AT THESE TWO SURFACES

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Although oestrogen is recognised to stimulate and suppress bone formation at cancellous and periosteal surfaces respectively, the basis for this differing action remains unknown. Emerging evidence suggests that angiogenesis plays an important role in bone formation, possible reflecting the involvement of shared endothelial-osteogenic precursors. We previously found that stimulation of cancellous bone formation by oestrogen involves expansion of the pool of osteoblast precursors within the marrow cavity, suggesting that bone formation at this site proceeds independently of angiogenesis. On the other hand, angiogenesis may play an important role in bone formation at the periosteum, which is not in direct contact with bone marrow. In the present study, we investigated whether differences in the role of angiogenesis in cancellous and periosteal bone formation explain the differential effect of oestrogen at these two envelopes. Angiogenesis was examined by evaluating VEGF expression with anti-VEGF-147 and anti-VEGF-165 antibodies, as well as CD31 expression (an endothelial cell marker), in mice (4/group) treated with 17beta-estradiol at 0.5mg/week. Animals were sacrificed 0, 1, 2, 4, 8 and 16 days after commencing treatment, following which tibiae were removed for sectioning, and bone histomorphometry and fluorescent immunohistochemistry were performed on paraffin-embedded and cryostat sections respectively.

Oestrogen rapidly stimulated new cancellous bone formation: Day0, 3.9+/-2.7; Day2, 11.6+/-3.6; Day4, 14.4+/-4.5; Day8, 14.9+/-5.0; Day16, 32.1+/-6.1 (data are BV/TV %; mean +/- SEM). No expression of VEGF was seen in the medullary cavity either before, at any time-point point after, oestrogen administration. In contrast, VEGF was expressed at relatively high levels within the periosteum, which appeared to be restricted to bone-forming surfaces, and to coincide with sites of CD31 expression. Furthermore, the level of periosteal VEGF expression was unaffected by oestrogen treatment.

We found that angiogenesis is associated with bone formation at periosteal, but not cancellous, surfaces. Hence, the differential response of cancellous and periosteal bone to oestrogen may reflect their differing dependency on angiogenesis. Since oestrogen did not appear to affect angiogenesis per se, we propose that this hormone suppresses periosteal bone formation by inhibiting osteogenic commitment and/or differentiation of common endothelial-osteogenic precursor cells within the periosteal vasculature.

P9

IMPAIRED GROWTH PLATE FUNCTION OF BMP-6 NULL MICE

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BMP-6 is known to be expressed by different skeletal cells including proliferating chondrocytes, suggesting a possible role in growth regulation. However, apart from small differences in bone size as measured by DXA, bmp-6-/- mice have previously been found to have a normal skeletal phenotype. In the present study, we explored the role of BMP-6 in regulating longitudinal growth, by examining whether growth plate function is impaired in bmp-6-/- mice. As part of this investigation, we analysed the response of bmp-6-/- mice to estrogen treatment, based on previous studies where we defined the dose-responsive inhibitory action of 17beta-estradiol (E2) on growth plate function in intact female mice.

Bmp-6-/- mice were crossed with resident C57Bl/6 mice, F1 hybrids inter-crossed, and homozygous bmp-6-/- mice and WT littermates generated. 10-week old female bmp-6-/- and WT mice were administered vehicle or E2 4, 40, 400, 4000 ug/kg/day by daily sc injection for 28 days (6-8 per group). Tibias were removed, and detailed histological analysis of the growth plates performed on longitudinal paraffin-embedded sections. Two-way ANOVA was subsequently performed to evaluate the effects of E2 treatment and genotype.

As previously found, E2 administration was associated with a dose-responsive decrease in proliferative zone cell number and width, and growth plate width ($P < 0.0001$). Interestingly, a wide range of growth plate parameters were reduced in bmp-6-/- compared to WT mice, with significant reductions observed on proliferative zone cell number and width, hypertrophic zone cell number and width, and growth plate width ($P < 0.001$). In addition, a dose-genotype interaction was observed, such that the highest dose of E2 was associated with a significant reduction in column density per mm in bmp-6-/- compared to WT mice (24.9+/-1.4 vs 32.9+/-1.7, $P<0.01$).

Although bmp-6-/- null mice have a relatively normal skeletal phenotype, when longitudinal growth is impaired by administration of estrogen, a clear deficit in growth plate function is apparent. Our results suggest that BMP-6 plays an important role in maintaining growth plate function in the presence of physiological inhibitors of this process, such as estrogen.

P10

SELECTIVE ESTROGEN RECEPTOR MODULATOR INHIBITS OSTEOCYTE APOPTOSIS DURING ESTROGEN LOSS. IMPLICATIONS FOR BONE QUALITY MAINTENANCE

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Ideally, Selective Estrogen Receptor Modulators (SERMs) have been designed to demonstrate all of the positive bone associated effects of estrogens without the estrogen induced soft tissue side effects. Estrogen exerts positive effects not only on the quantity but also on the quality of bone including the maintenance of osteocytes through the inhibition of their apoptosis. In this study, we have used a rat model of ovariectomy (OVX) to determine whether the osteocyte sparing effect of 17beta-estradiol can be mimicked by a Raloxifene analogue, the SERM LY 117018.

Sixteen juvenile female rats were divided into four treatment groups (n=3 per group): sham operated (SHAM), Ovariectomy (OVX), ovariectomy +17beta-estradiol (OVX+E2) and Ovariectomy + SERM (OVX + SERM). At 7 or 14 days following the start of treatment, the radius and ulna were removed. The percentage of apoptotic osteocytes, determined using an in situ nick-translation method, was increased (2.5 fold at 7 days and 6 fold at 14 days) in the OVX group compared with the SHAM operated group in the radii and ulnae, respectively. Treatment of OVX animals with either 17beta-estradiol (OVX+E2) at a dose rate of 0.125 mg/kg/day or LY 117018 (OVX+SERM) at a dose rate of 3 mg/kg/day prevented the increase in osteocyte apoptosis to a similar extent in both radius and ulna.

These observations demonstrate that in a similar way to 17beta-estradiol the LY 117018 SERM can exert a powerful inhibitory effect upon osteocyte apoptosis during estrogen loss pointing to the potential benefits of SERMs on both quantity and quality of bone in the post-menopausal individual.

P11

HIGH-DOSE OESTROGEN-INDUCED OSTEOGENESIS IS DECREASED IN AGED CBFA1 HETEROZYGOUS MICE

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Cbfa1 is a transcription factor critical in embryonic skeletal development, which also appears to be important in regulating

osteoblast function in the adult. In one study, young (10-week) cbfa1 heterozygous mice appeared to show a normal osteogenic response following bone marrow ablation, although aged mice (32-weeks) showed a markedly decreased response. However, in another study intramembranous bone formation was not impaired in 1-year old cbfa1 heterozygous mice during distraction osteogenesis. We have previously shown in mice that cbfa1 is up-regulated in a population of putative osteoprogenitor cells in the osteogenic response to high-dose oestrogen. The aim of the present study was therefore to determine whether haploinsufficiency of cbfa-1 occurs in the high-dose estrogen-induced osteogenic response in young and aged mice using, for the first time, a quantitative dynamic histomorphometric approach.

Young (10-weeks) and aged (26-weeks) cbfa1-heterozygous and wild-type female littermate mice were treated with vehicle or high-dose estrogen (0.5mg/animal/week) by subcutaneous injection for 4 weeks. Mice were then divided into 8 groups according to age, genotype and treatment with 6 animals per group. Following sacrifice, longitudinal tibial sections were prepared and examined by static and dynamic histomorphometry. Results were analysed by one-way ANOVA.

Estrogen treatment induced formation of new cancellous bone in both wild-type and cbfa1+/- mice. This occurred to the same extent in young mice of both genotypes. However, in aged cbfa1+/- mice this response was decreased by over 70% ($p<0.001$) when compared to aged wild-type mice. Significant reductions in cancellous double-labelled surfaces (dls/TV, 1.7+/-0.2 vs 1.0+/-0.4 mm2/mm3, $p<0.05$) and mineral apposition rate (1.8+/-0.1 vs 1.4+/-0.1 microns/day, $p<0.01$) were observed in aged cbfa1+/- mice compared to wild-types.

Aged cbfa1 heterozygotic mice display an abrogated osteogenic response to high-dose estrogen. We suggest that aged cbfa1+/- mice may eventually be capable of a full osteogenic response but haploinsufficiency initially leads to delayed bone formation. Further studies are required to determine the time course of this response and confirm whether aged cbfa1+/- mice can, given time, mount a comparable response to the one shown by wild-type mice.

P12
PHOTODYNAMIC THERAPY OF DISEASED BONE

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Photodynamic therapy (PDT) defines the oxygen-dependent reaction that occurs upon light-mediated activation of a photosensitizing compound, culminating in the generation of cytotoxic, reactive oxygen species, predominantly, singlet oxygen. We are investigating PDT treatment of diseased bone. Using a rat model of human breast cancer (MT-1)-derived bone metastasis we confirmed the efficacy of benzoporphyrin-derivative monoacid (BPD-MA)-PDT for treating metastatic lesions within vertebrae or long bones. Light administration (150 J) 15 mins after BPD-MA (2.5 mg/Kg, i.v.) into the lumbar (L3) vertebra of rats resulted in complete ablation of the tumour and surrounding bone marrow 48 hrs post-PDT without paralysis. Porcine vertebrae provided a model comparable to that of human for light propagation (at 150 J/cm) and PDT response (BPD-MA; 6 mg/m2, i.v.) in non-tumour vertebrae. Precise fibre placement was afforded by 3-D cone beam computed tomography. Average penetration depth of light was 0.16 ± 0.04 cm, however, the necrotic/non-necrotic interface extended 0.6 cm out from the treatment fiber with an average incident fluence rate of 4.3 mW/cm2. Non-necrotic tissue damage was evident 2 cm out from the treatment fiber. Current studies involving BPD-MA-PDT treatment of primary osteosarcomas in the forelimbs of dogs are very promising. Magnetic resonance imaging 24 hr post treatment reveal well circumscribed margins of treatment that encompass the entire 3-4 cm lesion. Finally, we are also interested in using 5-aminolevulinic acid (ALA) mediated PDT to treat osteomyelitis. Response to therapy was monitored as changes in bioluminescence signal of staphylococcus aureus (S. aureus)-derived biofilms grown onto 0.5 cm lengths of wire and subjected to ALA-PDT either in vitro or in vivo upon implant into the intramedullary space of rat tibia. Transcutaneous delivery of PDT (75 J/cm2) effectively eradicated S. aureus-biofilms within bone. Results support the application of PDT to the treatment of primary or metastatic lesions within bone. Secondly, that ALA-PDT may be useful as a treatment for osteomyelitis. Further studies aim to optimize the parameters of delivering PDT into bone and explore imaging technologies that can be used for clinical PDT.

P13
CARDIOVASCULAR DISEASE AND OSTEOPOROSIS: RELATIONSHIP BETWEEN HYPERTENSION AND FRACTURE

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Increased cardiovascular (CVS) risk has been clearly established in several rheumatic diseases, but osteoporosis is generally not included in this group.

Aim: To describe and quantify cardiovascular risk in a cohort of primary osteoporosis patients, and examine relationships with bone health

Methods: 90 primary osteoporosis patients (13 male, 77 female, median age 67.0, median years since diagnosis 4.0) were recruited, and had a range of CVS risk factors analysed, together with bone mineral density scan and bone health assessment.

Results: 45.5% of patients had received a cholesterol check, 67.7% a BP check and 31.1% a diabetes check in the last year.16.7% of patients had suffered from MI, angina or CVA, and 22.2% currently smoked cigarettes (30.0% ex-smokers).

7.7% had diastolic BP>90 mmHg, and 51.1% >80 mmHg. There was a significant difference between diastolic BP in those who had suffered a low trauma fracture compared with those who had not (median, 95%CI, 84.0 (80.0-88.0) v 70.0 (67.9-76.0) $p<0.001$). 8.9% had systolic BP>160 mmHg, and 36.7% >140 mmHg. There was a significant difference between systolic BP in those who had suffered a low trauma fracture compared with those who had not (median, 95%CI, 140.0 (130.0-144.0) v 124.0 (120.0-132.3) $p<0.001$). There was no relationship between use of antihypertensives and fracture or BP.

There was a relationship between BMD at the hip and HDL levels ($r=-0.29$, $p<0.05$), weight ($r=0.39$, $p<0.001$) and serum urate ($r=0.25$, $p<0.05$). 47.8% of patients had fasting cholesterol >5.2mmol/l, and the median was 5.35 (5.02-5.71). HDL levels were higher in those who took at least 20 mins exercise 3 times a week as compared with those who did not (1.24 (1.09-1.43) v 1.45 (1.33-1.58), $p=0.06$), and also correlated with serum urate ($r=-0.22$, $p<0.05$). 26.7% of patients took regular alcohol: they had significantly higher BMD at the spine compared with those who took none (0.955 (0.900-1.020) v 0.861 (0.814-0.930, $p<0.05$). Although alcohol consumption was higher in men ($p<0.01$), BMD at the spine was not. There was no relationship between alcohol and lipids or fractures.

Conclusion: There is a strong relationship between hypertension and fracture in this cohort of primary osteoporosis patients, which has important implications for fracture reduction. HDL levels appear linked to both BMD and exercise, and alcohol in this cohort is related to higher BMD at the spine. Cardiovascular and cerebrovascular disease are common.

P14
CYR61: A NOVEL INHIBITOR OF OSTEOCLAST FORMATION

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Multi-domain CCN proteins contain about 10% by mass cysteine residues and modulate cell proliferation, adhesion, migration, and differentiation. Many of the effects of CCN proteins appear to be due to binding to extracellular growth factors or integrins. CYR61 (CCN1) is expressed by a variety of tumour cell types and by proliferating osteoblasts, and appears to stimulate osteoblast differentiation. However, until now, the effect of CYR61 on osteoclasts was unknown.

In cultures of MCSF-dependent murine macrophages treated with 100ng/ml RANKL for 5 days, addition of 100-1000ng/ml recombinant human CYR61 caused a significant and concentration-dependent decrease in the number of multinucleated osteoclasts (without evidence of increased cell death), together with a parallel reduction in the level of TRAP activity. Inhibition of osteoclast formation was confirmed by RT-PCR, since CYR61 markedly reduced the expression of the osteoclast phenotypic markers TRAP, MMP-9, calcitonin receptor and cathepsin K. Similarly, CYR61 inhibited osteoclast formation in 1,25(OH)2vitaminD3-stimulated cultures of rabbit bone marrow and in RANKL-stimulated cultures of human MCSF-dependent human monocytes. However, CYR61 did not affect the formation of multinucleated cells when added to osteoclast precursors

prior to fusion, or affect the number or resorptive activity of long bone-derived rabbit osteoclasts cultured on dentine discs, indicating that CYR61 affects early osteoclast progenitors but not differentiated osteoclasts.

To study the mechanism underlying the inhibitory effect of CYR61, we examined its effect on RANKL-induced signalling. CYR61 did not affect RANKL-induced phosphorylation of p38 or ERK1/2, or RANKL-induced activation of NFATc1, in MCSF-dependent osteoclast precursors. Furthermore, despite its known ability to bind to alphavbeta3 and other integrins, CYR61 did not appear to affect integrin-induced phosphorylation of ERK1/2 following adhesion of osteoclast precursors onto osteopontin.

These observations demonstrate that CYR61 is a hitherto unrecognised inhibitor of osteoclast formation, although the exact mechanism of inhibition remains to be determined. Together with its ability to stimulate osteoblasts, CYR61 could represent an important bifunctional local regulator of bone remodelling.

P15

ABNORMALITIES OF CHONDROCYTE GROWTH IN-VITRO IN HEREDITARY MULTIPLE EXOSTOSIS

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Introduction: In hereditary multiple exostosis (HME) the synthesis of the polysaccharide heparan sulphate (HS) is disrupted. HS-proteoglycans are low affinity receptors involved in fibroblast growth factor signaling. Activation of FGF receptor 3 (FGFr3) on mature chondrocytes leads to growth attenuation rather than stimulation. We tested the hypothesis that in HME chondrocytes with absent or reduced HS-PG synthesis there is impaired FGFr3 ligand binding and loss of control of chondrocyte proliferation.

Materials and methods: Chondrocytes were harvested from normal growth plate (epiphyseodesis) or HME osteochondroma cartilage cap obtained as surgical discard and cultured to 70% confluence in growth media. Cells were re-plated for experimentation. Growth curves were obtained for cells over a period of 5 days. In addition proliferative responses of healthy and HME chondrocytes were determined after low serum synchronization followed by challenge with either FGF 2 or FGF 9 (10 and 100ng/ml) and incorporation of BrdU for 2hours every two hours over a twenty eight hour period. Using these techniques it is possible to describe in detail the time dependent entry of cells into S-phase of the cell cycle and compare cell lines and treatment.

Results: Significant differences were observed in the growth characteristics over a five-day period ($p<0.05$). Under baseline growing conditions the chondrocytes derived from osteochondroma had a more rapid doubling time when compared with the normal growth plate chondrocyte (2.6days+/- 0.6 vs 4.9days+/-1.0, $p<0.05$). In response to incubation with FGF-9 cells from normal growth plate had a lower peak proportion of cells entering the s-phase than with media alone (7% vs 25%). This inhibition was not observed in chondrocytes from osteochondroma. Both primary cell lines exhibited an increased peak proportion of cells entering the s-phase in response to FGF-2.

Conclusions: These results indicate significant differences in the in-vitro growth characteristics of chondrocytes derived from the cartilage caps of osteochondroma when compared to cells derived from the normal growth plate. It would appear that osteochondroma chondrocytes are resistant to the normal regulatory effect of FGF-9 on cell proliferation. The differential response to FGF-9 may be responsible for the growth differences observed both in-vitro and in-vivo.

P16

INHIBITING ADIPOCYTE DIFFERENTIATION IN HUMAN PREADIPOCYTES AND FETAL FEMUR-DERIVED MESENCHYMAL STEM CELLS USING SMALL INTERFERING RNA

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RNA interference (RNAi) is an evolutionary conserved post transcriptional mechanism of gene silencing triggered by double-stranded RNA. Human mesenchymal stem cells have the ability to differentiate into osteogenic, chondrogenic, reticular, myoblastic and adipogenic cell lineages offering a unique cell source for tissue engineering/regeneration strategies if appropriate strategies to manipulate these lineages can be derived. The current study has examined the potential of small interfering RNAs (siRNA) against human peroxisome proliferator activated receptor gamma (PPARgamma) to suppress adipocyte differentiation in human preadipocytic cells from subcutaneous tissue and fetal femur-derived (8-12 weeks) mesenchymal cells. Human preadipocytes were transfected using PPARgamma specific siRNAs (PPARgamma-siRNA) using a liposome-based strategy, which was verified and optimized with a fluorescence-labeled siRNA. Adipocyte quantification after 11 days showed a significantly lower adipocyte number in human preadipocyte cell cultures transfected with PPARgamma-siRNA1 (63% lower, $p<0.01$) or PPARgamma-siRNA2 (68% lower, $p<0.01$) compared to adipogenic controls. In support of a reduction of PPARgamma 1 and 2 mRNA levels following transfection with PPARgamma siRNA, similar reductions in human AdipoR1, AdipoR2 and glycerol 3-phosphate dehydrogenase were observed in the human preadipocyte cultures as analysed by reverse transcription PCR. Examination of oil-red O concentrations in PPARgamma-siRNA1 and PPARgamma-siRNA2 cultures showed a 57% and 77% decrease, respectively, while no significant differences were observed in the Lipofectamine or negative siRNA controls, compared to the adipogenic control. Examination of human fetal femur-derived mesenchymal populations' transfected with PPARgamma-siRNA1 or PPARgamma-siRNA2 displayed significantly decreased fatty acid binding protein 3 (FABP3, an adipocyte specific marker) expression, and reduced oil red O concentration by 38% and 39%, respectively, while no significant difference was seen in the negative siRNA control, compared to the adipogenic control. A critical role of PPARgamma in adipogenesis was verified in human preadipocytes and mesenchymal stem cells. In conclusion, these studies show the efficacy of PPARgamma siRNA strategies to inhibit adipocyte differentiation in human preadipocytes and human fetal femur-derived mesenchymal populations providing a useful model to investigate stromal cell plasticity and differentiation.

P17

EPIGENETICS OF OSTEOARTHRITIS: IS DNA DMETHYLATION INVOLVED IN THE PATHOGENESIS OF THE DISEASE?

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Osteoarthritis (OA) is a disease of unknown cause that progressively worsens. One key feature is failure of the cartilage matrix due to degradation by proteases that are derived from the synovium or so-called 'degradative' chondrocytes present in the superficial zone of OA cartilage, but absent from the deep zone of low-grade OA or control cartilage. Degradative chondrocytes multiply and the abnormal enzyme expression is transmitted to the daughter cells. Because these enzymes are silenced in normal articular chondrocytes and the abnormal expression in degradative chondrocytes is heritable, we hypothesized that this abnormal gene expression has resulted from epigenetic 'unsilencing' via demethylation of DNA that is normally methylated. To test this hypothesis, we selected MMP-3, -9, -13 and ADAMTS-4, typically expressed by degradative chondrocytes, and identified in the promoters the so-called 'CpG sites', where the methyl-group is added to cytosine. These promoters were 'sparse' CpG promoters (did not contain a CpG island), a condition that may favour pathological demethylation.

Methylation status was correlated with mRNA (RT-PCR) and protein (immunocytochemistry) expression. RNA or DNA were extracted from articular cartilage of control, or deep and superficial zones of OA patients. Higher mRNA expression of all proteases was found in the superficial zone compared with deep-zone OA or control cartilage. Only 51% of CpG sites were methylated in superficial-zone OA chondrocytes vs 80% in control or deep-zone OA chondrocytes. Not all CpG sites were equally susceptible to demethylation, as some were uniformly methylated, whereas methylation was absent in others. For each protease there was at least one critical CpG site, which was methylated in most controls and un-methylated in most OA patients, suggesting these sites are crucial for silencing. Other studies have shown that demethylation at a single CpG is sufficient

for un-silencing, but this may also result from overall hypo-methylation. The current studies provide evidence that the abnormal expression of degradative enzymes by superficial-zone OA chondrocytes (as shown by RT-PCR and immunocytochemistry) correlates with DNA demethylation. In other words, abnormal enzyme expression is an epigenetic event. Because epigenetic changes may be reversible, these changes could become a target for therapeutic intervention, especially early in the disease.

P18

RISEDRONATE PREVENTS BONE DISEASE AND REDUCES TUMOUR BURDEN IN A SYNGENEIC MODEL OF MULTIPLE MYELOMA

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Multiple myeloma is a B cell malignancy resulting in the monoclonal proliferation of plasma cells in the bone marrow. A major clinical feature is the development of a devastating bone disease mediated by increased osteoclastic activity and a reduced osteoblastic response. Compounds capable of altering the bone marrow micro-environment and inhibiting bone resorption, such as bisphosphonates, are likely to be effective in preventing myeloma bone disease. In the present study we have investigated the effect of risedronate and the phosphonocarboxylate analogue NE10790 on myeloma bone disease in the 5T2MM syngeneic model of myeloma. Injection of 5T2MM cells into C57BL/KaLwRij mice resulted in the development of bone disease characterised by an increase in osteoclast numbers, a decrease in bone area and the development of osteolytic lesions. Treatment of mice with risedronate or NE10790, from the time of tumour cell injection, prevented osteoclast formation (Vehicle = 13.88±2.31%; risedronate = 0.46±0.26%; NE10790 = 1.37±0.44%), prevented the 5T2MM induced decrease in bone area (Vehicle = 0.96±0.44%; risedronate = 10.21±2.14%; NE10790 = 5.66±1.04%) and completely prevented the development of bone lesions (Vehicle = 6.2±1.39%; risedronate = 0.33±0.24%; NE10790 =1.1±0.41%). Risedronate but not NE10790 treatment was associated with a decrease in tumour burden. In conclusion, risedronate and NE10790 prevent the development of myeloma bone disease in the 5T2MM model. Risedronate treatment was associated with an anti-tumour effect that maybe independent of the effect on bone resorption.

P19

THE LETROZOLE (L), EXEMESTANE (E), AND ANASTROZOLE (A) PHARMACODYNAMICS (LEAP) TRIAL: A DIRECT COMPARISON OF BONE BIOCHEMICAL MEASUREMENTS BETWEEN AROMATASE INHIBITORS (AIS) IN HEALTHY POSTMENOPAUSAL WOMEN

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It has been suggested that there are differences between steroidal AIs (E) and non-steroidal AIs (A and L) on bone turnover, with steroidal AIs having a less negative effect. The LEAP trial is an open, randomized pharmacodynamic study comparing the effects of the three AIs on safety parameters, including serum markers of bone turnover, in healthy postmenopausal women with normal bone mineral density at the spine and hip.

Healthy volunteers from centres in the UK and Hungary were randomized to receive A (1 mg/day), L (2.5 mg/day), or E (25 mg/day) orally, once daily for 24 weeks. Changes from baseline in log transformed bone ALP, serum C-telopeptide crosslinks (CTX), parathyroid hormone (PTH) and propeptide of type I procollagen (PINP) at 24 weeks on A, were compared with those on L and E by ANCOVA, adjusting for treatment, baseline measurement, BMI, smoking status and baseline oestradiol. No adjustments were made for multiple comparisons.

A total of 102 healthy volunteers were recruited, with 90 participants evaluable at 24 weeks (29 A, 29 L, 32 E). Participant demographics were similar between the treatment groups in terms of age, years since menopause, and history of hysterectomy and oophorectomy. Estimated means were calculated from the back transformation of the least squares means from ANCOVA. There were similar increases in the bone turnover markers at 6 months in response to anastrozole, letrozole and exemestane; the estimated mean changes (95% CI) for alkaline phosphatase were +1.9(-4.3,+8.6),+2.9(-2.9,+9.1) and +6.6(+0.8,+12.7),and for PINP were +13.6(+3.0,+25.3), +11.4(+1.8,+21.8) and +23.5(+13.3,+34.6), respectively and for CTX were +16.6(+2.9,+32.2), +27.7(+13.9,+43.2) and +23.1(+10.3,+37.4). The estimated mean percentage change (95%CI) from baseline in serum PTH was -7.6(-18.0,+4.0), -10.7(-19.9,-0.4) and -20.5(-28.4,-11.8)for anastrazole , letrozole and exemestane respectively. There were no statistically significant differences between the AIs, with the exception of PTH, which showed a greater decrease in PTH with E than with A (p=0.04).

We conclude that the steroidal and non-steroidal AIs appear to have similar effects on markers of bone turnover. All three licensed AIs result in increases in bone turnover.

P20

OSTEOCLASTOGENESIS AND OSTEOCLAST ACTIVITY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

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During treatment for childhood acute lymphoblastic leukaemia (ALL), it is possible that increased osteoclastogenesis and/or osteoclast activity contributes to the observations of increased serum markers of bone resorption and reduced bone mineral density (BMD). The role of the leukaemia and its treatment in this process is, however, currently unclear. Using peripheral blood mononuclear cells (PBMCs) and our established method of in vitro osteoclastogenesis, we have compared normal childhood osteoclastogenesis and osteoclast activity with that during treatment for childhood ALL.

PBMCs were obtained from 15 normal children; age 2-15, mean 8.7 years (control group), and 16 children with ALL; age 2-16, mean 6.8 years (ALL group). Samples were obtained from the control group on a single out-patient visit. Those from the ALL group were taken on up to twenty occasions throughout treatment with the initial sample at diagnosis where possible. The additional samples were scheduled during blocks of chemotherapy (time of bone marrow suppression) and subsequent bone marrow recovery before the next block of chemotherapy. Cells were seeded onto coverslips (CS) and dentine slices (DS), and cultured with M-CSF (25 ng/ml) and RANKL (30 ng/ml) for 14 days (CS) and 21 days (DS) respectively. Osteoclasts were defined as multinuclear (3 or more), TRAP positive cells and functionality was demonstrated by DS pit formation. Results are expressed as % resorption DS area.

Samples from normal children yielded functional osteoclasts in all cases (% resorption area 5 to 90), whereas samples from children with ALL did not always yield functional osteoclasts (% resorption area 0 to 49.2 ± 14.6). In samples obtained from 5 out of 7 subjects with ALL, osteoclastogenesis was not possible at diagnosis or in the first two weeks of treatment.

Our results suggest that children undergoing initial chemotherapy for ALL have impaired osteoclastogenesis partly as a consequence of disease-induced suppression of stem cells and this may take time to recover following initiation of anti-leukaemic therapy.

P21

VALIDATION OF CORTICAL THICKNESS ESTIMATES IN THE FEMUR MADE WITH WHOLE BODY COMPUTED TOMOGRAPHY (QCT) AND MINDWAYS SOFTWARE: COMPARISON WITH HIGH RESOLUTION PQCT

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Background: Mayhew et al(1) showed that female femoral neck cortical thickness declined supero-laterally by about 50% in adulthood. This makes the elderly femoral neck vulnerable to cortical buckling in a sideways fall. However, it is unclear that thin cortices of 1-1.5mm thick can be measured usefully in vivo with whole body

QCT. Method: Seven femurs from the Melbourne femur collection were scanned both on the Densiscan pQCT (0.275 mm voxel edge); and on a Siemens Sensation 16 (0.580mm) using a water bath. The Densiscan cortical thicknesses had been cross-calibrated against histological measurements(2). Cortical cross sections were compared after reconstruction in the same plane normal to the femoral neck axis, at Kuijper's definition of minimum area. Each imaged cortex was divided into 16 sectors subtended from the centre of area and the mean thickness of the cortex for each sector calculated using Mayhew's equations(1). The histological thicknesses for each sector (estimated from the pQCT data) were regressed on the Siemens estimates to calculate root mean square (RMS) errors of estimation from Siemens scans. Results: Using untransformed data, the residuals always were non-normally distributed (Shapiro-Wilk test) violating the condition for valid regression. This was corrected by taking logs, which also reduced the absolute size of the uncertainties of estimation of thin cortical thicknesses. The regression equation relating simulated in vivo results to our 'gold standard' estimates of true cortical thicknesses had an R square of 0.70 and a RMS error of 0.12, equivalent to an uncertainty (coefficient of variation for a sectoral thickness) of about 30%. Conclusions: Using this combination of hard- and soft-ware, valid estimates of cortical thickness can be made on single slices of the femoral neck that should be useful in clinical studies. Increased precision is likely to come from reconstructing across several slices along the neck axis, as described by Mayhew et al(3) . Refs: 1 Mayhew PM et al Lancet 366:129-35; 2005. 2 Crabtree N et al J Bone Miner Res 16: 1318-28; 2001. 3 Mayhew PM et al Bone 34:352-61; 2004.

P22

A DXA-BASED COMPOSITE BEAM MODEL OF THE PROXIMAL FEMUR FOR STRESS ESTIMATION

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The DXA-based models have been developed to assess stress of the proximal femur. Beck's curved-beam model (Beck et al, Invest Radiol 25:6; Mourtada et al, J Orthop Res 14:483) assumes that the femur is composed of a fully mineralised bone, and the variation in BMD is accounted for by variable pixel thickness. This results in a stress distribution independent of BMD. We recently developed a composite beam model that treats the femur, similar to the 2D finite element (FE) model (Testi et al, Ann Biomed Eng 30:80), as a plate of constant thickness and of different materials. The stress can be calculated using the classic equations for composite beam. This study compares the performance of the beam models against the FE model assuming this is the 'gold standard'.

Models simulating lateral falls were generated from CT scans of the both proximal femurs of 27 women with osteoporosis (mean age 81.1, range 65-86 yr) recruited at a single centre participating in the HORIZON-PFT Study. Simulated DXA scans were generated by summing up voxel density in the A/P direction. For each simulated DXA scan, a 2D FE, a curved-beam and a composite beam model was generated. Bone material was assumed to be isotropic elastic and its elastic modulus dependent on density (Keyak et al, J Biomech 31:125). The differences in stress at each pixel, the root mean square (RMS) of the stress differences, and the differences in peak positive and negative stresses were calculated between the beam models and FE model.

The maximum differences in normal, shear and first principal stresses and the RMS of their differences, the differences in peak normal and principal stresses between our model and FE model were all significantly (paired t-test, $P<0.05$) smaller than those between the curve-beam and FE model. The opposite was true for the maximum differences in second principal stress and its RMS, and the differences in peak shear stress.

In conclusion, our composite beam model appears to produce stress distribution more realistic than the curved beam model and may be useful in clinical assessment of the risk of osteoporotic hip fracture.

P23

FACTORS AFFECTING THE COHESION OF IMPACTION BONE GRAFT

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Aim: In vitro study looking at factors that influence the cohesive properties of mixes for impaction grafting. Factors include use of pure bone, graft extenders (solid and porous), bone washing and the addition of clotted blood.

Methods: 16 groups of samples produced using fresh frozen femoral heads, graft extender, and clotted blood. Samples impacted then loaded on servo-hydraulic testing machine. The cohesion of each sample was calculated from the stress/strain diagrams produced.

Results: Pure bone has significantly higher cohesion than when mixed with extender ($P<0.001$). Pure graft extender has zero cohesion. Washing bone chips had no significant effect on the cohesion. Adding clotted blood significantly increased the cohesion of pure bone graft($P<0.019$) and samples with a 50/50 mix of bone and porous graft extender ($P<0.054$).

Conclusions: Graft extender is less cohesive than bone so is harder to work with. Handling of porous extender when mixed with bone can be improved with the addition of clotted blood.

P24

INFLUENCE OF BIRTH WEIGHT ON PQCT MEASUREMENTS OF CORTICAL BONE SITES IN YOUNG GAMBIAN ADULTS

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Birth weight has been shown to predict overall skeletal size independently of postnatal factors. However, it is not known whether birth weight and current weight predict different aspects of bone structure in adulthood. In this study, we investigated the influence of birth weight and current weight on cortical and medullary area at skeletal sites rich in cortical bone.

Total cross-sectional bone area (BA), cortical area (CrtA), medullary area (MedA), total bone mineral content (BMC), cortical BMC, total volumetric bone mineral density (vBMD) and cortical vBMD were determined at three sites (66% from distal radius and ulna in the upper limb, and 50% from distal tibia in the lower limb) using peripheral quantitative computed tomography (Stratec, XCT 2000), in 46 women and 64 men aged 17-21 years, from a rural village in The Gambia. Birth weight (BWt) was obtained from clinic records.

In univariate and multiple regression analysis, both BWt and current weight (Wt) were independent positive predictors of BA at the radius and tibia in males and females ($p<0.05$). In males, BWt also predicted MedA ($p<0.01$), but not CrtA, while Wt predicted CrtA in both sexes (males: $p<0.001$, females: $p<0.05$) but not MedA. BWt was not a significant predictor of BMC in either sex. As a consequence, BWt was a negative predictor of total vBMD ($p<0.01$) and of cortical vBMD ($p<0.05$) in males. Current weight was a positive predictor of BMC and cortical BMC in both sexes ($p<0.01$), but not of vBMD or cortical vBMD. Age and height were included as possible confounders but had little effect on the results.

These results agree with previous studies in Caucasians showing that birth weight is a determinant of skeletal size in adulthood. Our results also suggest that birth weight and current weight are independently associated with different aspects of skeletal size, with cortical area influenced by factors in early adulthood and medullary area influenced by prenatal factors. Trabecular and cortical regions of bone may be programmed differently during prenatal and postnatal life. Future work will extend this analysis to include skeletal sites rich in trabecular bone.

P25

MATERNAL 25(OH)-VITAMIN D STATUS IN LATE PREGNANCY PREDICTS INTRAUTERINE BONE MINERAL ACCRUAL IN THE OFFSPRING

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Recent evidence suggests that intrauterine bone mineral accrual predicts osteoporosis risk in later life, and that maternal 25(OH)-vitamin D status in pregnancy is a determinant of childhood bone mass at age 9 years. In this study, we aimed to explore the relationship between intrauterine bone mineral accrual in the offspring and maternal 25(OH)-vitamin D status during late pregnancy.

Pregnancies were recruited from the Southampton Women's Survey, a unique, ongoing, well-established cohort of women, aged 20-34 years, assessed before and during pregnancy. Maternal 25(OH)-vitamin D status was measured by radio-immunoassay in late pregnancy (34 weeks); the healthy, term, neonates underwent whole body (WB) DXA within 20 days of birth, using a Lunar DPX instrument. T-tests were used to compare bone mass in offspring of mothers who were deficient vs replete in 25(OH)-vitamin D during late pregnancy.

556 (286 male) neonates were studied. Offspring of mothers who were deficient (<33 nmol/l) in 25(OH)-vitamin D in late pregnancy had lower bone mass than those of replete mothers. Thus the mean WB bone area (BA) of the female offspring of 25(OH)-vitamin D deficient mothers was 110cm2 vs 119cm2 in offspring of replete mothers (p=0.04). The mean WB bone mineral content (BMC) for offspring of deficient vs replete mothers was 58g vs 63g (p=0.04) respectively. The relationships in the boys did not reach statistical significance. There was no association with maternal alkaline phosphatase.

These data are consistent with previous findings that mothers deficient in 25(OH)-vitamin D in pregnancy have children (at age 9 years) with reduced bone mass. Our current study indicates that this association is present at birth, and we hypothesise that modulation of calcium transport across the placenta, perhaps via an active calcium transport protein, might underlie this process. Further elucidation of these mechanisms may allow development of novel therapeutic strategies to optimise childhood bone mineral accrual and thus reduce osteoporotic fractures in future generations.

P26

THE CONTRIBUTION OF FEMORAL NECK TRABECULAR BONE TO HIP FRACTURE PREVENTION

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Fractures of the proximal femur have more impact than any other osteoporotic fracture. The mechanism of fracture in a sideways fall is controversial. Lowered femoral bending resistance was not a major effect of ageing in the studies of Yoshikawa or Looker and Beck. Mayhew et al (Lancet 366:129;2005) studied a representative sample of female femurs ex vivo across a wide age range and analysed the femoral neck critical stresses at which buckling in compression was predictable. The mechanism they proposed was disputed by Bouxsein and others (ibid pp1523-) because of the support the cortex receives from sub-cortical trabeculae. We have therefore estimated the size of the anti-buckling effect of the trabeculae attributable to their resistance to cortical sideways displacement (the initial event in buckling) in the same 35 femurs studied by Mayhew et al. Each was scanned with the Densiscan pQCT (pixel edge 0.275mm) as described previously and the cancellous bone area as %bone+marrow (CnBAr) was calculated from the mean density of the trabecular compartment (as % of maximum density of bone adjusted for the proportionately lower density of trabecular bone - from Loveridge and Boyde Bone35:929;2004). The equations of Hernandez & Carter (Bone 29:74;2001) were used to calculate individual cancellous elastic moduli. In an extension of Mayhews analysis, long established structural theory (Hetenyi M: Beams on Elastic Foundations;1946, Ch7 p148) was used to show that trabeculae increase the critical stress as a predictable square root function of cortical thickness and the ratio cancellous:cortical moduli. From these considerations, the trabecular contribution to critical stress was 24% of total (median); but from age 80 critical stress was still predicted to decline dangerously close to the elastic limit for cortical bone (leading to brittle behaviour in compression, negating its materials toughness). In multiple regression analysis the trabecular contribution was positively related to the cortical contribution to critical stress (F=23.7 p<0.0001). It was inversely related to cortical surface curvature independently (F=18.0 p<0.0002). We hypothesise that the trabecular contribution to preserving critical stress could be enhanced through repetitive mechanical loading microscopically vibrating flatter thin cortices and their interconnections with little effect on DXA BMD.

P27

THIAZIDE DIURETICS DIRECTLY STIMULATE OSTEOBLAST FUNCTION

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Thiazide diuretics are commonly used for the treatment of hypertension and their use has been associated with an increased bone mineral density in patients undergoing such a therapy. The common target of this treatment is the thiazide-sensitive sodium-chloride cotransporter (NCC), which is expressed in the kidney. It has been proposed that, acting on the kidney NCC, thiazides produce a positive calcium balance and drive the observed bone-sparing effect. However, a direct effect of thiazides and the expression of NCC in bone cannot be ruled out and, indeed, have been hypothesized.

We have previously reported the presence of NCC mRNA and protein in the cells of osteoblastic origin in freshly frozen undecalcified preparations of human and rat bone, as well as in tissue culture models of osteoblasts [1].

Here we detected the ability of human osteoblast-derived cells (MG63) and fetal rat calvarial cells (FRC), to proliferate and differentiate in the presence or absence of metolazone, a thiazide-like diuretic.

Chronic metolazone treatment (1-100 microM) dose-dependently promotes the expression of osteoblast differentiation markers, Runx2 and osteocalcin, measured by western blotting, in the absence of a proliferative effect (n=3; p<0.05). In FRC cells, metolazone also dose-dependently increases the formation of mineralized nodules, quantified by von Kossa staining (n=6; p<0.05). These findings are specific to thiazide diuretics and are not reproduced using the loop diuretic bumetanide. Furthermore, in MG63 cells, small interfering RNA designed against human NCC decreases the expression of Runx2.

Taken together, these results provide strong evidence for a direct action of thiazide diuretics on bone, through the thiazide-sensitive sodium-chloride cotransporter. Our findings support the view that thiazides should be considered as the diuretics of choice in elderly hypertensive patients.

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P28

ASSOCIATION BETWEEN TGF-BETA AND LRP 5 & 6 GENETIC POLYMORPHISMS WITH FRACTURE RISK AND BONE MINERAL DENSITY IN THE EPOS STUDY

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The nature of the genetic component in the determination of osteoporosis and fracture risk largely remains to be demonstrated

given the diversity of the results reported by published studies evaluating various candidate gene polymorphisms. Some previous work has suggested that the TGF-beta and LRP 5 & 6 genetic polymorphisms are associated with bone mineral density (BMD) and/or fracture risk.

We therefore investigated this hypothesis using data from 4,217 men and women aged 20-95 years (mean=66 SD=11) from 26 centres across Europe (13 countries) in the European Prospective Osteoporosis Study (EPOS), of which 1,217 were fracture cases and 3,495 had hip and/or spine BMD measurements by DXA. Fracture cases were matched to up to 3 controls from the same centre, sex, and age (90% within +/-6 years). Five TGF-beta polymorphisms TGF-b (800, 509, Leu10Pro, Arg25Pro, and Thr263Ile) and 2 LRP 5 (Val667Met & Val1330Ala) and 1 LRP 6 (Ile1062Val) polymorphisms were determined by PCR genotyping (Taqman) in a central laboratory. Conditional logistic regression was used to model association with any fracture and analysis of variance to assess BMD differences between genotypes.

None of the polymorphisms evaluated showed convincing evidence of association with risk of any fracture or BMD levels. Although the rare homozygotes (AA) for the TGF-beta 800 polymorphism demonstrated significantly increased risk (P=0.010), OR =3.09 95% CI (1.30, 7.31) compared to common homozygotes (GG), the frequency of the AA genotype was only 0.71% (n=30/4217) in this data suggesting a potentially unreliable result. Similarly the LRP 5-Val667Met polymorphism showed increased risk (P=0.031) of any fracture OR=2.03 (1.15, 16.17) for rare (AA) compared to common (GG) homozygotes but the frequency of the AA genotype was only 0.26% (n=11/4217). Femoral neck and trochanter BMD were significantly lower in heterozygotes (GA) than common homozygotes (GG) for the two LRP 5 polymorphisms, but BMD levels in the rare AA were not different from the common GG.

The results suggest that the evaluated genetic polymorphisms are unlikely to be associated with fracture risk or BMD levels and that large-scale genetic association studies are required to reliably assess any associations conferred by rare genotypes.

P29
PAGET'S DISEASE: A CONFORMATIONAL DISEASE?

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Paracrystalline inclusions are known to occur in the nuclei and cytoplasm of osteoclasts in Paget's disease (PDB) and in sporadic inclusion body myositis (s-IBM) a late-onset degenerative muscle disease. In s-IBM inclusions have features of aggresomes, aggregates of misfolded proteins that assemble when a cell's ability to degrade misfolded proteins is overwhelmed. Aggresomes contain proteasomal subunits, gamma tubulin, heat shock proteins, ubiquitin, the 'offending', misfolded protein and are surrounded by an extensive network of intermediate filaments, used to transport proteins to the aggresome. s-IBM has an unknown aetiology, but is now recognised to be one of a growing number of 'conformational diseases'. Given the ultrastructural similarities of the inclusions seen in PDB and s-IBM and the frequent mutation in PDB of p62, a ubiquitin-binding protein involved in proteasomal degradation, we investigated the expression of p62 and proteasome-associated proteins in bone biopsies of patients with PDB. In PDB, osteoclasts expressed high levels of p62 in distinct areas of the cytoplasm, and intranuclear with a localisation similar to that of ultrastructural inclusions. The pattern of cytoplasmic staining for p62 was similar to that seen for the 20S proteasomal alpha and beta subunits. In addition we found clear staining of selective osteoclast nuclei for the 20S beta subunit. Ubiquitin staining was seen in both the cytoplasm and in selective osteoclasts nuclei and, similar to the 20S beta subunit, but unlike p62, this staining was throughout the whole nucleus. Ultrastructural analyses revealed that in PDB osteoclasts intracytoplasmic inclusions, identical to intranuclear inclusions, were frequently seen and we suspect that these correlate with the regions containing high levels of p62 and proteasomal subunits. Overexpression of p62 in HEK293 cells resulted in formation of aggresomes surrounded by large areas of intermediate filaments, a feature also seen in cells overexpressing the mutated form of RANK that leads to early onset PDB and a feature seen in some osteoclasts in PDB biopsies. We conclude that there are similarities between s-IBM and various forms of PDB and suggest that defective protein degradation may form part of the pathogenesis of PDB. Paget's disease may therefore be a 'conformational disease'.

P30
VITAMIN D STATUS AND BONE HEALTH IN YOUNG BRITISH AND MIDDLE EASTERN WOMEN

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The relative contributions of dietary sources and sunlight exposure to vitamin D status are generally unknown. There is no dietary reference value (DRV) for vitamin D in the UK and vitamin D insufficiency is becoming a common occurrence, especially since scares of contracting skin cancer following sunlight exposure have been highlighted. In recent years other countries have redefined their recommended dietary intake values for vitamin D, e.g. the USA adequate intake (AI) recommendation for 0-50 year olds is now set at 200IU/d. In Middle Eastern Countries there is currently no public health policy for vitamin D food fortification/supplementation in existence despite evidence of extensive vitamin D deficiency.

The aims of this study were to determine the extent of vitamin D insufficiency in premenopausal women living in the UK and Saudi Arabia and to assess the impact of dietary and lifestyle habits on indices of bone health.

A total of 275 young women from the UK and 100 healthy premenopausal from Jeddah, Saudi Arabia were recruited. Mean age was 25.4 [2.8] and 23.1 [3.5] years in the two population groups. A fasted blood sample was taken for assessment of vitamin D status (measured by HPLC). BMD was assessed at the lumbar spine and femoral neck using DXA. All subjects were interviewed concerning their habitual dietary intake, physical activity levels and general lifestyle.

Mean values for 25(OH) D were 25.6 [12.6] ng/ml in the UK women (n=99) and 8.9 [3.6] ng/ml in the Saudi Arabian women. Over 80% of the Middle Eastern women had 25(OH) D levels below 12ng/ml compared with 11% of UK women. Over 60% of UK women had vitamin D insufficiency (25OHD below 30ng/ml). All women were below 5ug/d for vitamin D intake. Further analysis of the UK dataset are required but these data suggest that a dietary recommendation for vitamin D is urgently needed in the UK and a public health policy is crucial in countries where mode of dress restricts exposure to sunlight.

P31
PRENATAL INFLUENCES ON SKELETAL GROWTH ASSESSED BY 3D ULTRASOUND AND DXA

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Epidemiological studies suggest that a woman's nutrition, body build and lifestyle during pregnancy influence the skeletal development of her offspring, initiating long-term effects on bone structure and fracture risk in late adult life. To study prenatal influences on fetal skeletal growth we have developed a new technique to measure fetal femur volume using a Kretz-Voluson 3D ultrasound machine. Examining the reproducibility of fetal femur volume measurements, at 19 weeks gestation the standard deviation (SD) of the intra-operator differences was 0.056 cc, compared with a between-subjects SD of 0.185 cc; at 34 weeks, the SD of the intra-operator differences was 0.473 cc and the between-subject SD was 1.351 cc. This suggests that measurement error is small relative to the biological variability between fetuses. Within a population-based study of women's nutrition and health before and during pregnancy (the Southampton Women's Survey) we measured femur length and volume in 517 fetuses at 19 and 34 weeks gestation, and examined maternal influences on fetal femur size and growth velocity. 285 of these fetuses also had DXA measurement of neonatal bone mineral content (BMC), and we examined the relations between fetal femur growth and neonatal BMC.

Adjusting for gestational age, we found that maternal height was not associated with fetal femur size at 19 weeks, but was associated with femur length and volume at 34 weeks (r=0.20, p<0.001 and r=0.11,

p=0.02, respectively). Maternal height was also associated with greater femur length growth velocity between 19 and 34 weeks (r=0.12, p=0.009) but not volume growth velocity (r=0.04, p=0.4). Maternal BMI, age and parity were not associated with femur size or growth velocity, but femur length growth velocity tended to be lower in smoking mothers (p=0.07). Femur size at 19 weeks was not associated with neonatal BMC, but BMC was higher in fetuses with greater femur length and volume at 34 weeks (r=0.23, p<0.001 and r=0.23, p<0.001, respectively). Femur length and volume growth velocity between 19 and 34 weeks were also positively associated with neonatal BMC (r=0.13, p=0.03 and r=0.13, p=0.04, respectively). These findings support a critical period for skeletal growth between 19 and 34 weeks gestation.

P32

DISTINCTIONS IN MINERAL MORPHOLOGY AND MAGNESIUM AND SILICON CONTENT BETWEEN OSTEOPOROTIC AND OSTEOARTHRITIC BONE

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Skeletal structural properties are determined by bone quality as well as mass, and a central factor is the nature of the inorganic phase. Differential calcification of hard and soft connective tissues influences the functioning of bones and joints, and modulations in mineral character and location may contribute to disease states.

Normal porcine and osteoporotic (OP) and osteoarthritic (OA) human femoral heads were examined optically and ultrastructurally with special attention to the topography of the calcification front beneath the articular cartilage. After chemical digestion of articular cartilage, FEGSEM and EDX microanalysis provided insight into the complex substructure and elemental composition of the calcification front. In addition, mineral objects liberated from more mature bone regions were similarly examined.

Populations of mineral microspheres were the predominant feature of all specimens. In the normal animal and elderly OP femoral heads these were assembled along a smooth, discrete, undulating calcification front, as regular particles of approximately 1 micron diameter, with a substructure of clusters of sinuous calcified filaments, 5 nanometres wide, composed of 'chains of beads'. In contrast, in the elderly OA femoral heads, the microspheres were disordered and diffusely distributed, encroaching beyond the calcification front, and ranging in size from 1 to 4 microns diameter. They apparently arose within the many mineral-loaded chondrocytes, and their ultrastructure was coarsely granular relative to the finely beaded filaments. Moreover, in OA the Ca:P ratio was closer to crystalline hydroxyapatite than in OP and healthy bone, and it also lacked their distinctive magnesium and silicon peaks, indicating a potential dysfunction in the process of mineral formation and its control in OA.

It was concluded that significant differences in the distribution and morphology of mineral microspheres between OP and OA may have implications for mechanical stiffness, fracture predisposition and pathogenesis.

P33

VERTEBRAL FRACTURE AND NON-OSTEOPOROTIC VERTEBRAL DEFORMITY: RELATIONSHIP WITH BONE DENSITY AND CLINICAL CRITERIA ASSOCIATED WITH OSTEOPOROSIS

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Accurate diagnosis of osteoporotic vertebral fracture is complicated by the presence of non-osteoporotic deformities that appear to have 'reduced' vertebral height. Our hypothesis is that osteoporotic vertebral fracture involves depression of the central endplate: visual identification of vertebral fracture may be improved by excluding deformities that do not exhibit depression this feature. The aim of this study was to determine whether VF and non-osteoporotic deformity identified using this approach are associated with osteoporosis.

We studied spinal radiographs for 904 postmenopausal women (from a population enriched with VF cases) using the approach described above. These were categorised as i) normal, ii) non-osteoporotic deformity (non-ODf) (including non-osteoporotic VF) or iii) osteoporotic vertebral fracture. We tested associations between diagnostic categories and bone mineral density (BMD), height, weight, age, height loss since age 25, low-trauma fracture history and kellgren scores for degenerative change. We also compared values after subdividing women in the non-ODf group according to quantitative morphometry (QM): these were the QM-positive group (women with VF identified by QM in the same vertebrae) and the QM-negative group (with no QM fractures).

A total of 231 women (26%) were diagnosed with osteoporotic VF compared to 344 (38%) identified by QM. Non-ODf was identified in 342 women (38%) and 330 women (37%) were diagnosed normal. Women with VF were older, with lower height, weight and height loss than those categorised normal or non-ODf. Values were similar for women with QM-positive and QM-negative non-ODf. Diagnosis of VF was associated with history of low-trauma non-vertebral and vertebral fracture (odds ratios for VF were 2.6 and 9.3 respectively). Higher kellgren scores were associated with diagnosis of non-ODf (odds ratio = 3.3). Age-adjusted SD units (z scores) were lower than expected among women with VF (but not those with non-ODf) for BMD measured at the femoral neck and lumbar spine (total hip BMD did not differ). The z scores for women with non-ODf were similar in the QM-positive and QM-negative groups.

Conclusion: non-osteoporotic deformity without evidence of central endplate fracture in postmenopausal women is not associated with low bone density or other clinical criteria commonly associated with osteoporotic VF.

P34

CRYOPRESERVED AORTIC ALLOGRAFTS VERSUS SYNTHETIC MEMBRANES FOR LONG-BONE GUIDED TISSUE REGENERATION

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Background and objective: In guided tissue regeneration a membrane is used for defect isolation to protect it against invasion from surrounding tissues and to keep intrinsic healing factors 'in situ'. This technique has been successfully used in maxillo-facial surgery, but short experience has been reported in long-bone defects, with synthetic membranes and with variable results. In the other hand, calcification and ossification inside the arterial wall have been described. The aim of the study was to evaluate the use of cryopreserved aorta allografts as membranes for guided tissue regeneration in comparison with expanded poly-tetra-fluoro-ethylene (e-PTFE) synthetic membranes.

Methods: Prospective, randomized, blinded study in 15 New-Zeland rabbits. 10 mm mid-diaphyseal defects were created in both radii: 10 defects were covered with a cryopreserved aortic allograft as a tube, 10 with an e-PTFE membrane and 10, with no barrier membrane, served as controls. Animals sacrifice at 6-12-24-30 months. Studies: X-rays, CT, MR, morpho-densitometric analysis, electronic and optical microscopy. Immuno-cytochemistry on tissues and arterial wall cells cultured.

Results: None of the control defects healed. Nine defects covered with an artery completely reconstituted, but only six of those covered with e-PTFE, with a nearly normal cortical-medullar pattern and with progressive increasing in density and thickness of medullar and cortical to values similar to those of the normal bone. Histological studies showed no inflammatory response to the arterial graft, direct union between the artery and the regenerated bone and even mature bone between the elastic laminae of the arterial wall, suggesting superior biocompatibility properties. Immuno-cytochemistry and ultrastructural studies suggest that arterial allografts could act not only as membrane barriers, with additional osteoinductive properties due to trans-differentiation of viable arterial wall cells (endothelial, smooth muscle and/or tissue specific stem cells) towards osteoblastic cells, and also due to ossification secondary to changes in proteins of the arterial extracellular matrix. This could be the application of the process of arterial wall calcification and ossification (usually seen in arteriosclerosis, gender, diabetes or kidney failure) for regeneration of long-bone defects.

Conclusion: Cryopreserved aortic allografts can be used as membrane barriers for guided bone regeneration, with superior results to e-PTFE membranes.

P35

BONE DISTRIBUTION IN THE CROSS SECTIONS OF THE FEMORAL NECK AND INTERTROCHANTERIC REGIONS: A STUDY USING CLINICAL QCT

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Recent data (Mayhew et al, 2005, Lancet 366: 129) supported the suggestion that the thinning of the posterior part of the superolateral cortex of the femoral neck (FN) with age contributes to hip fracture during sideway fall. The purpose of this study is to see if clinical QCT can be used to study this topic.

We analysed the CT scans of both proximal femurs of 27 women (mean age 81, range 65-86 yr) with osteoporosis recruited at a single centre participating in the HORIZON-PFT study. Cortical bone was assumed with density >= 0.3g per square centimetre. The femurs were digitally rotated to a standard orientation thus ensuring a consistent cross section (CS) definition. The FN and inter-trochanteric (IT) axes were determined and the CSs defined along the axes with a spacing of about 1.5 mm. Each CS was divided into 8 sectors with the centre at the bone mass centre: Inferior, Infero-Anterior, Anterior, Supero-Anterior, Superior, Supero-Posterior, Posterior, Infero-Posterior. Bone area (BA), bone polar moment of inertia (PMI) and cortical shell thickness (CST) were calculated for the whole CS and each octant. The results presented here relate to CSs at the start, middle and end of FN, and at the middle and end of IT.

One-way ANOVA of the middle FN data showed that the largest BA and CST were in octant I, minimum BA in octant A, S, S-P and P, and minimum CST in octant A, S-A, S and S-P. Two-way ANOVA of the data showed that CS and octant position and their interaction were significant factors affecting the parameters. However, if the parameters were expressed as the % of their CS values, the CS position was no longer significant. Scheffe post-hoc comparison revealed that the octant I had the largest BA and CST, while octant A, S-P and P had the lowest BA and octant S-P and P the thinnest cortex.

In conclusion, this study used clinical QCT and was able to confirm the findings of micro CT on excised samples: the thinnest part of the proximal femur is the posterior octant.

P36

COMPARISON BETWEEN DENSITOMETRIC VERTEBRAL FRACTURE ASSESSMENT AND ASSESSMENT OF SPINAL RADIOGRAPHS

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Improvements in image quality have led to increased uptake of densitometric vertebral fracture assessment (VFA). In previous studies, VFA and radiological assessment of spinal radiographs have been compared for the diagnosis of osteoporotic vertebral fracture (VF), but there has been little focus on the causes of discrepancies between the two techniques. The aims of this study were to 1) compare agreement between visual readings of radiographs and VFA scans and 2) identify sources of discrepancy between methods.

Spinal radiographs and VFA images were performed in 203 women ages 65 and older (mean 75 years) referred for bone densitometry. Two observers (R1and R2) independently read all images using a modified visual approach, reading VFA images first and blinded to each other's results. Vertebrae were classified as follows: i) definite fracture; ii) non-osteoporotic deformity; iii) uncertain fracture; iv) not analysed (inadequate image quality) and v) normal. Categories ii) through iv) were regarded as negatives.

We considered the reading of radiographs by R1 as the gold standard; using this approach the prevalence of VF was 15.3% (31 women). These women had lower bone mineral density (BMD) compared to those without VF (BMD T scores were -2.06, -1.88 and -2.24 at the lumbar spine, total hip and femoral neck compared to -0.99, -1.12 and -1.49 in women without VF ($p < 0.01$). Interobserver agreement was better for radiographic readings (kappa, k = 0.74 compared to 0.65 for VFAs). Agreement between readings of radiographs (R1) and VFA scans was good for both observers. For VFA (R1), k = 0.60 and for VFA (R2), k = 0.58. Of 6 false positives for VFA (R1), 4 were classified 'uncertain VF' from radiographs. Re-examination of VFA (R1) suggested these may be mild fractures. There were 13 false negatives for VFA (R1), of which 10 were 'uncertain VF'. Exclusion of the vertebrae classified 'uncertain' improved agreement between VFA and radiography (for R1, k = 0.86).

Conclusion: agreement between imaging techniques was good. Vertebrae classified 'uncertain' represented the main cause of discrepancy between methods.

P37

DAY-TO-DAY VARIABILITY OF CIRCULATING OSTEOCLAST PRECURSOR POPULATIONS IN POSTMENOPAUSAL WOMEN

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Osteoclasts are derived from mononuclear cell precursors of the monocyte/macrophage lineage. Osteoclast precursor cells (OCP) are present in the peripheral circulation and have been characterised by their expression of CD14 and M-CSFR. The number of OCP and their receptor expression may be quantified by flow cytometry. It is unclear whether the evaluation of OCP by these methods is reproducible enough to use in a clinical investigation and whether cell number is related to circulating concentrations of estradiol or bone resorption markers.

The aims of the study were a) to determine the day to day variability in the number and M-CSFR receptor expression of OCP b) to examine the relationship between OCP number and M-CSFR expression and serum 17beta-estradiol and C-telopeptide collagen crosslinks (CTX).

We studied 27 postmenopausal osteopenic women ages 56 to 74 years mean (SD), 62 (4.25) and 5-33 years since menopause. Peripheral blood was drawn on 2 occasions, with a 7-14 day interval. OCP were identified using fluorescent labelled antibodies (CD14-FITC and M-CSFR-PE). Stained cells were analysed by flow cytomentry (Becton Dickinson FACScaliburTM). 17beta-estradiol and CTX were measured in serum by immunoaassy. Least significant change (LSC) was calculated as follows

LSC=coefficient of variation (CV) x 1.96 x SQRT2.

The mean (SE) M-CSFR fluorescence intensity was 54.8 (8.6) on visit 1 compared to 54.5 (7.4) on visit 2. The percentage of CD14+ cells also M-CSFR+ was 83.1(4.5) on visit 1 and 88.7 (3.5) on visit 2. Serum estradiol was 15.4 (1.42) pg/mL and 15.9 (1.46) pg/mL on visits 1 and 2 respectively, whilst serum CTX was the same for both visits 0.59 (0.04) microg/mL. There was no correlation between the number of OCP or their M-CSFR expression and serum 17beta-estradiol or CTX.

The CV (LSC) of M-CSFR fluorescence intensity (19.7% (54.4%)) and of the percentage of CD14+/M-CSFR+ cells (12.1% (33.6%)) are similar to those for 17beta-estradiol (14.8% (41.0%)) and CTX (12.2% (33.8%)). This suggests that the use of flow cytometry measurements to identify OCP may be reproducible enough for use in a clinical investigation; larger studies are needed to evaluate the relationship to sex hormones and bone turnover markers.

P38

QUALITY OF LIFE AND CURRENT PAIN LEVELS ARE ASSOCIATED WITH FRACTURE RATHER THAN WITH OSTEOPOROSIS

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Pain and morbidity are known to be associated with osteoporotic fractures. The aim of this study was to investigate the association between quality of life, current pain and previous self-reported fracture.

Bone mineral density (BMD) measurements were obtained at the lumbar spine, neck of femur and total hip using a Hologic QDR 4500 (Bedford, MA) on 80 patients. All patients also completed a visual analogue scale (VAS) to indicate current levels of pain and the Quality of Life (QoL) questionnaire of the European Foundation for Osteoporosis (QUALEFFO-41)(Lips et al, 1991).

Individuals were then grouped into osteoporotic (n = 48) and normal / osteopenic (n = 32) based on their T-score at any site (Hologic spine

and NHANES III hip reference data). No significant differences were found between the two groups for total QoL (osteoporotic: mean total score = 32.10, SD = 19.39, normal / osteopenic: mean total score = 29.96, SD = 18.13, p = 0.30) or for VAS scores (osteoporotic: mean score = 3.07, SD = 3.45, normal / osteopenic: mean score = 2.42, SD = 2.95, p = 0.17).

When subdividing individuals according to a previous history of self-reported low trauma fracture (fracture group: n = 40, no-fracture group: n = 40) the mean total QoL score for the fracture group was 35.96 (SD = 18.8) and the no-fracture group was 23.45 (SD = 15.16). The mean VAS score was 3.44 (SD = 3.47) for the fracture group and 1.53 (SD = 2.46) for the no-fracture group. There is a significant difference between those individuals with a self-reported history of fracture compared with those individuals who have not experienced previous fracture in terms of both quality of life (p = 0.0009) and VAS score (p = 0.003).

These results support the theory that reduced quality of life and increased pain are associated with fractures rather than with osteoporosis itself. Further research is required to investigate the degree of pain and QoL reduction due to complications of osteoporotic fracture.

Lips et al, Osteoporosis Int 1999;10:150-160

P39

MMP-2 AND MMP-9 SERUM VALUES IN OSTEOPOROTIC SUBJECTS UNDERGOING MUD PACK TREATMENT

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Introduction: The etiology of osteoporosis is multifactorial: several factors are involved. Osteoblasts and synoviocites release factors such as metalloproteases, which are (are essential in normal development and remodelling and are capable of degrading bone matrix and to influence bone resorption (Lhotaks et al 2000; Ichinose et al 2000, Dequeker et al. 2003).

It is now known that MMP-9 is implicated in the regulation of chondrogenic and osteogenic cell differentation during early stages of repair and in vivo studies have demonstrated as lack of MMP-9 provokes delayed unions of fractures by persistent cartilage at the injury site, while recent findings suggest thet bisphosphonates inhibit bone resorption by abrogating MMP-2 action (Colnot C et al, 2003).

Aim of the present study is investigate if mud pack therapy, a biologic treatment , frequently used in patients suffering from cartilage degenerative diseases, influences biochimical parameters involved in bone resorption as MMP-2 and-9.

Materials and methods: we enrolled 20 osteoporotic subjects (mean age 58.7 ± 4.3) undergoing a cycle of 12 mud pack treatments and collected their blood samples before and after the end of the therapy to assay MMP-2 and MMP-9 serum values.

Both the enzymes have been assayed by quantitative sandwich ELISA, recognizing the active natural human enzyme form (Quantikine pro-MMP-2 and MMP-9 immunoassay, R and D System Inc., Minneapolis, MN, USA).

Results: our results show a decrease in MMP-2 serum levels (ng/ml) (244.3 ± 23 before and 227.7 ± 16,7 after the treatment) and an increase in MMP-9 values (ng/ml) (420.9 ± 21.5 before and 543.8 ± 29.9 after the therapy). Both the results are statistically significant (p <0.01).

Conclusions : these results allow to hypotize an interaction of MPT with the bone metabolism. The decrease in MMP-2 serum values could be considered as a possible modulation of bone catabolism, while MMP-9 increase could mean an influence on bone reparative process in adults.

These results encourage further studies to understand if MPT could be considered , with the pharmacologic therapy and the physical exercise, beneficial for osteoporosis prevention in peri menopausal women and to improve the bone reparative processes in osteoprotic fractures.

P40

GLUCOCORTICOIDS STIMULATE P21 EXPRESSION IN GROWTH PLATE CHONDROCYTES

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Recent evidence suggests that cell cycle genes play an important role in the coordination of chondrocyte proliferation and differentiation. The inhibitory effects of glucocorticoids (GCs) on chondrocyte proliferation are consistent with GCs disrupting cell cycle progression and promoting cell cycle exit. However, the underlying mechanisms by which GCs alter cell cycle control in chondrocytes are unknown. Progression of the cell cycle is promoted by the activation of cyclin-dependent kinases (CDKs). Cyclin-dependent kinase inhibitors (CDKIs) play an important role in maintaining growth arrest and cell differentiation by binding to, and inactivating CDKs. Recent reports have indicated that some CDKIs may force cells to exit from the cell cycle and differentiate, and others have shown that expression of the CDKI p21(CIP1/WAF1) is increased in terminally differentiated cells. In this study we examined the possibility of p21 mediating Dex-induced growth retardation. Female mice were injected daily with 5mg/kg Dex for 7 days. At day 7, body weight, body and tibiae length in Dex treated mice were significantly reduced compared to untreated mice (14.8%, 7.5% and 4.6% respectively; p<0.05). Within the proximal tibiae, the epiphyseal growth plate width in Dex mice was significantly less than controls (18.1%; p<0.05), due to a significant reduction in the width of both the proliferative and hypertrophic zones (20.1% and 15.2% respectively; p<0.05). p21 protein was expressed at higher levels in the hypertrophic chondrocytes of the growth plate which is consistent with its role in mediating cell cycle exit. Quantification of the effects of Dex on p21 gene and protein expression was examined in the murine ATDC5 chondrogenic cell line. Increased expression of p21 mRNA and protein was observed during chondrocyte differentiation, whereas expression of other CDKIs such as p15, p18, p19, p27 and p57 remained unchanged. After exposure to Dex (10-6M) for 6 or 12h, p21 mRNA and protein expression was increased. This data suggests that Dex increases the p21 chondrocyte expression, which may promote cell cycle exit and, in part, explain the growth retardation and altered growth plate morphology noted in Dex treated mice.

P41

THE ANTI-PROLIFERATIVE AND PRO-APOPTOTIC EFFECTS OF CERAMIDE ON GROWTH PLATE CHONDROCYTES

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Abnormal growth patterns are commonly observed in children suffering from chronic inflammatory diseases. These disorders are associated with the increased pro-inflammatory cytokine production, which have been shown to inhibit growth plate chondrocyte proliferation and induce apoptosis. Ceramide, a sphingosine-based lipid second messenger, mediates many of the actions of pro-inflammatory cytokines. Ceramide inhibits IGF-1 signalling and induces apoptosis in numerous cell types. It is likely that ceramide mediates at least some of the actions of TNF alpha, IL-1 beta and IL-6 on bone growth. Therefore in this study we have determined the effects of C2-ceramide a cell permeable ceramide analogue, on the growth of murine chondrogenic ATDC5 cells and fetal metatarsals from 18 day-old mouse embryos. In ATDC5 cells, C2-ceramide significantly induced apoptosis at both 40mM (82%; P<0.05) and 25uM (53%; P<0.05). At 40uM, ceramide significantly reduced proliferation (62%; P<0.05). Cell number was also significantly reduced at both 40uM (72%; P<0.05) and 25uM (62%; P<0.05). C2-ceramide did not markedly alter the mRNA expression of markers of chondrogenesis and differentiation (sox 9, collagen II, aggrecan and collagen X). Further studies investigated whether C2-ceramide was exerting it's anti-proliferative and pro-apoptotic effects though inhibition of IGF-1 signalling. In ATDC5 cells, C2-ceramide (25uM) +IGF-1 (24h, 100ng/ml) induced a 68% reduction in proliferation (P<0.001 versus control). However, ceramide -IGF-1 induced a comparable 61% decrease (P<0.001 versus control). C2-ceramide (40uM) induced a 31% reduction in the growth of fetal metatarsal cultured for 8 days in the presence and absence of IGF-1 (100ng/ml; P<0.001). AG1024 (10uM), an IGF-1 and insulin receptor blocker significantly reduced ATDC5 proliferation (28%, P<0.001). C2-

ceramide (25uM) significantly reduced proliferation compared to AG1024 treatment (55%, P<0.001). C2-ceramide and AG1024 in combination further reduced proliferation compared to C2-ceramide alone (46 %; P<0.01). C2-ceramide (25uM) treatment (24h) did not alter IGF-1-stimulated (10min, 100ng/ml) phosphorylation of insulin receptor substrate-1 (IRS-1), Akt (protein kinase B) or Erk 1/2. In conclusion, C2-ceramide inhibits proliferation and induces apoptosis in growth plate chondrocytes and impairs bone growth, through an IGF-1 independent mechanism.

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EFFECTS OF SYNOVIAL-FLUID AND SERUM FROM CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS ON GROWTH PLATE CHONDROCYTE DYNAMICS

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Children with juvenile idiopathic arthritis (JIA) suffer from a range of growth disorders. Pro-inflammatory cytokine concentrations within serum and synovial-fluid are altered in JIA patients and may interact directly with epiphyseal chondrocytes and modulate growth. To test this hypothesis we exposed cultured murine fetal metatarsal bones to synovial-fluid and serum samples from JIA patients. Paired samples of serum and synovial fluid were obtained from 4 patients with JIA (Child 1:systemic JIA;Child 2,3,4: oligoarticular JIA). Serum samples were obtained from a further 4 patients. Child 5,6,7: polyarticular JIA; Child 8 oligoarticular JIA). Median age was 7.3 yrs, median height SDS was -0.22 and median Height Velocity SDS over the 6 months prior to sample collection was 0.27. Six children were pre-pubertal. Concentrations of IL-1 beta and TNF alpha were determined in the serum and synovial fluid, and metatarsals were cultured in serum free medium for a 9-day period in the presence of the samples (1:10 dilution). At day 9, all serum samples reduced metatarsal growth by a median of 28% (range, 13,42). Only synovial fluid from Child 1 and Child 2 reduced metatarsal growth (43.2% and 16.1% respectively; P<0.05). There was no correlation between Height SDS or Height Velocity SDS and metatarsal growth. Further studies of chondrocyte dynamics were performed with the synovial fluid from Child 1 (4.62yrs; Height SDS,-1.73; Height Velocity SDS,-0.53; prepubertal). Metatarsal proliferation (determined by [3H] thymidine uptake) was significantly reduced (66%; P<0.001) following exposure to synovial fluid for the 9-day period. Concentrations of IL-1 beta and TNF alpha were above average (27 pg/ml and 137 pg/ml) in the synovial fluid (98 pg/ml and 529 pg/ml respectively). Metatarsals were subsequently exposed to the synovial fluid in the presence of neutralising antibodies to either IL-1 beta or TNF alpha (10ug/ml). However, no significant changes in metatarsal growth were observed in the presence of these antibodies. This study demonstrates that both synovial fluid and serum JIA samples are able to impair linear bone growth. Our studies show that neither IL-1 beta nor TNF alpha in these fluids play a predominant individual role at the level of the growth plate.

P43

THE DEVELOPMENTAL EXPRESSION OF PHOSPHO1 PRIOR TO SKELETAL MINERALISATION AND ITS PRESENCE WITHIN MATRIX VESICLES

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PHOSPHO1 is phosphatase that has previously been implicated in generating inorganic phosphate for matrix mineralisation. Despite its localisation to mineralising tissues, little is known about its expression in relation to the onset of mineralisation during embryonic development or in adult bone. It has also yet to be demonstrated whether PHOSPHO1 is present within matrix vesicles (MVs) and if expression is developmentally regulated. Whole-mount in situ hybridisation indicated that PHOSPHO1 expression was found in all chick long bones. Expression occurred prior to E6.5 and was initially restricted to the mid-shaft of long bones but by E11.5 expression was observed over the entire length of the diaphysis. Alcian blue/alizarin red staining revealed that PHOSPHO1 expression preceded the deposition of mineral suggesting that it is involved in the initial events of mineral formation. Immunohistochemical staining of E6.5 tibia mirrored the in situ hybridisation results and revealed that the PHOSPHO1 staining was localised to the osteoid and associated periosteal osteoblasts. The expression of PHOSPHO1 preceded the mineralisation of the osteoid as assessed by von Kossa staining which is known not to occur until E7.5 in the chick. Expression of PHOSPHO1 was observed in osteons within the cortical bone of the 1 day and 3 week-old chicks. Staining mirrored that of calcein fluorescence. Further compelling evidence that PHOSPHO1 plays a role in mineralisation comes from its presence in medullary bone but not cortical bone of adult 2 year-old chicks. In adults, bone apposition has ceased and no mineralisation at the periosteum takes place. However, medullary bone in adult female birds constantly undergoes remodelling as illustrated by calcein fluorescence. Isolated MVs from growth plate chondrocytes contained high levels of PHOSPHO1 as determined by immunoblotting. In addition, expression of PHOSPHO1, like alkaline phosphatase activity was found to be upregulated in MVs isolated from chondrocytes induced to differentiate by the addition of ascorbic acid. This suggests that both enzymes may be developmentally regulated. These studies provide for the first time direct evidence that PHOSPHO1 is present in MVs and is its developmental expression pattern is consistent with a role in the early stages of matrix mineralisation.

P44

A NOVEL ELECTROPORATION METHOD FOR EFFICIENTLY TRANSFECTING HUMAN OSTEOCLASTS

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The study of osteoclast biology has been hampered by the lack of non-viral methods available to transfect these cells. We have recently developed an electroporation-based protocol for efficient transfection of human M-CSF-dependent osteoclast precursors, which subsequently differentiate into mature, resorbing osteoclasts by treatment with RANKL. After Lymphoprep gradient isolation, human peripheral blood mononuclear cells are seeded into 75cm^2 flasks (12-15x10^6 per flask) in alphaMEM (+10% FCS, 100U/ml penicillin, 0.1mg/ml streptomycin, 2mM L-Glutamine, 20ng/ml recombinant human M-CSF). On day 4 the cultures are washed with PBS to remove non-adherent cells and the medium replenished. 100ng/ml rhRANKL is added when the adherent cells reach 80% confluence, typically day 6. 24-48 hours later, the osteoclast precursors are removed by trypsinisation and resuspended at 1x10^6 cells/100microlitre Mouse Macrophage Nucleofector Solution (Amaxa). 2micrograms of endotoxin free dsDNA (eg pCMV-GFP) is added to the cell suspension prior to electroporation in the Amaxa NucleofectorII unit. Immediately after transfection, cells are diluted to the required seeding density in RPMI medium containing 20% FCS and cooled on ice for 5-10 minutes. The medium is then supplemented with RANKL and M-CSF, prior to seeding onto dentine discs in 96-well plates (2x10^4 cells/well). The following day, medium is replaced with alphaMEM + 10% FCS + RANKL/M-CSF. Multinucleated, TRAP-positive, vitronectin receptor-positive human osteoclasts form within 2 days of transfection, and resorption of dentine by transfected osteoclasts can be detected by day 4. Up to 50% of the osteoclasts that form express the transgene, and expression is usually retained for at least 7 days. We have begun to use this approach to study the subcellular localization of EGFP-tagged small GTPases in osteoclasts. For example, EGFP-tagged Rab6 could be detected in osteoclasts and localised to the Golgi, as expected. Treatment of these cells with NE10790, a specific Rab GGTase inhibitor that prevents the prenylation and membrane localisation of Rab proteins, completely disrupted the Golgi-association of Rab6. This novel transfection method is therefore an effective and simple new approach for expressing DNA constructs in human osteoclasts, and will enable the localisation and role of specific proteins in this cell type to be studied more easily.

P45

THE IDENTIFICATION OF TRANSCRIPTIONAL TARGETS ASSOCIATED WITH OSTEOBLAST APOPTOSIS SIGNALING IN RESPONSE TO GROWTH FACTOR WITHDRAWAL

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It has recently been reported that osteoclast apoptosis in response to M-CSF withdrawal is critically regulated in vivo and in vitro by the pro-apoptotic protein Bim, which accumulates in response to decreased survival kinase signalling. We report that osteoblastic cell lines also respond to mitogen withdrawal by upregulating Bim. Withdrawal of serum or reduction to 1% FCS triggers mass apoptosis within 24h in MBA-15.4 mouse osteoblasts, assessed by TUNEL, ICC detection of PARP cleavage, acridine orange and DAPI staining. Bim protein levels are very low in unstressed cells but begin to accumulate from 2h following serum reduction, reaching a peak between 8-24h. Caspase 3 activity increased strongly from 4-8h. The upregulation of Bim at 8h is blocked by co-treatment with either actinomycin D or cycloheximide, indicating rapid, de novo protein synthesis. In haemopoietic lineage cells, Bim is reported to be constantly degraded by the proteasome following phosphorylation by survival kinases, ERK and PKB/Akt. We find in osteoblasts that treatment with the proteasome inhibitor MG132 results in accumulating levels of Bim protein, suggesting that Bim is also regulated by the proteasome in osteoblasts. Other cell stressors which reduce survival kinase signalling such as detachment or treatment with high-dose glucocorticoids also trigger upregulation of Bim in MBA 15.4 osteoblasts. Interestingly we found using Real Time Quantitative RT-PCR that the FLICE inhibitory proteins, cFLIPS and cFLIPL, that act as inhibitors of pro-caspase 8 in the Fas- and integrin dysfunction- induced apoptotic pathways, were upregulated in response to 20% serum stimulation. This increase was suppressed by co-treatment with the MEK inhibitor, U0126, implicating ERK signaling in FLIP transcriptional regulation in osteoblasts. Multi-BH domain pro-apoptotic members Bak and Bax were abundant in osteoblast and remained unchanged during 24 h of serum reduction. Unlike osteoclasts, osteoblasts depend for their survival on multiple growth factors. However, withdrawal of serum depletes the majority of growth factors in culture and we report that osteoblasts respond to this by upregulating Bim and undergoing apoptosis.

P46

AMYLOID PRECURSOR PROTEIN CLEAVAGE IN OSTEOBLASTS GENERATES THE INTRACELLULAR SIGNALING PEPTIDE AICD AND A NOVEL EXTRACELLULAR ADHESIVE FRAGMENT

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Gamma-secretase targets transmembrane proteins releasing cleaved fragments into the extracellular space and signaling peptides into the cytoplasm, where they translocate to the nucleus to modify gene transcription. Substrates of gamma-secretase include key molecules involved in osteoblast differentiation and survival, such as N-cadherin and Notch. Amyloid precursor protein (APP) is another common target for gamma-secretase, however the role of APP in the skeleton is unknown. Cleavage of APP by gamma-secretase results in the release of A-beta peptides into the extracellular space (accumulation of which causes neurodegeneration in Alzheimer's disease) and also produces an amyloid intracellular domain (AICD) that binds the nuclear adapter protein Fe65 forming a transcriptionally active 'AFT' complex through interaction with nuclear Tip60. We have determined the expression and function of gamma-secretase cleavage of APP during in vitro osteogenesis of human mesenchymal stem cells (MSCs). Expression of gamma-secretase components APH 1alpha, APH-1beta, nicastrin, PEN-2, Presenilin1 and Presenilin2 was demonstrated in MSCs by both qRT-PCR and western blot analysis. Using a specific fluorimetric assay we observed a significant increase in gamma-secretase activity during osteogenesis, corresponding with elevated alkaline phosphatase activity. The predominant isoform of APP identified by RT-PCR in osteoblasts differed to that found in neuronal tissues: osteoblastic-APP contained a Kunitz Inhibitor domain that biases APP cleavage towards production of the more amyloidogenic forms of A-beta peptides to promote amyloid plaque formation. Western blot analysis of osteoblasts identified a novel 55kDa APP C-terminal fragment containing the A-beta domain in addition to full length APP. The AICD cleaved signaling product of APP was also identified in osteoblasts and we used a specific chemiluminescent immunoassay to confirm production of secreted A-beta40. The expression of both Fe65 and Tip60 was also shown in osteoblasts by RT-PCR and immunoblotting. Osteoblasts showed a significant increase in adhesion to matrices containing aged A-beta plaques compared to non-aged A-beta peptide controls and Congo red staining of adult rat ulnae identified amyloid deposition in osteoid seams.

These data provide the first evidence of active gamma-secretase and APP processing in osteoblasts, supporting a role for A-beta plaques in cellular adhesion and suggesting broader AFT-dependent gene regulatory activity.

P47

INTRAUTERINE PROGRAMMING OF SKELETAL DEVELOPMENT: A LONGITUDINAL STUDY

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Epidemiological studies suggest skeletal growth is programmed during intrauterine and early postnatal life. We hypothesize that age-related decrease in bone mass has, in part, a fetal origin and are investigating this using a rat model of maternal protein insufficiency.

Dams received either 18% (control) or 9% (low protein) diet during pregnancy, and the offspring (n=139) studied at selected time points (4, 8, 12, 16, 20, 47, 75 weeks).

The growth trajectory was significantly reduced in female offspring in the restricted group at all timepoints studied from 4 weeks through to 75 weeks. In contrast, male offspring in the restricted diet group showed a modulated growth trajectory only between 4 to 8 weeks of age. Femora from males in the restricted diet group were longer than controls at 47 weeks of age. Restricted diet group females had shorter tibiae at 12 and 20 weeks of age. In the restricted diet groups, when normalized to mass, females showed longer tibiae and femora per unit mass at 12 weeks, whereas males showed longer femora per unit mass at 12 weeks and shorter at 20 weeks.

No differences were observed between control and low protein groups in bone mineral content or bone mineral density in the skull, vertebrae, femur, or tibia in 2 males+females per diet group per timepoint (4-47 weeks) using a PIXImus bone densitometer. We are now extending this study using micro-CT analysis of 75 week samples. Analysis of serum showed a significantly lower level of IGF-1 in female offspring in the restricted diet group at 4 weeks of age. In addition, osteocalcin levels were significantly lower in both males and females in the restricted diet group at 4 weeks of age. 25-OH vitamin D was significantly lower in 'restricted' males at 8, 12 and 20 weeks of age.

These results indicate a variety of affects of a maternal low protein diet on the development of bone structure and function. Clearly, there is a need to understand the key role of the nutritional environment in early development on programming of skeletal development and its implicit consequences in later life.

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CHARACTERIZATION OF $P2X_7$ RECEPTOR EXPRESSION AND FUNCTION IN HUMAN OSTEOBLASTS

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The $P2X_7$ receptor ($P2X_7R$) is the largest member (595 aa) of the purinergic ligand-gated cation channels, whose natural ligand is ATP. It is known to regulate osteoclast formation and activity, and is thought to play a significant role in skeletal response to mechanical loading. Very little, however is known of its precise action in osteoblasts. We have studied $P2X_7R$ expression and function using two cell lines representing different stages of osteoblast differentiation (MG63, early; SaOS2, late).

$P2X_7R$ expression was confirmed in both cell lines using western blotting (approximately 65 kD), immunocytochemistry (more intense labelling in nucleus compared to cytoplasm), and RT-PCR, with significantly higher levels in MG63 cells. Quantitative real time RT-

PCR indicated that receptor expression increased with cell density. $P2X_7R$ splice variants recently reported (Cheewatrakoolpong B, et al, 2005) in other cell types were not detected. ATP (0.001 - 0.4 mmolar) significantly reduced the cell number in a concentration-related manner after 2, 5, and 7 days. For example, MG63 and SaOS2 cell number was reduced with 0.4 mmolar by approximately 12.8%, and 29.7% respectively after 5 days. However, using the same concentrations and time course, ATP significantly stimulated alkaline phosphatase (ALP) activity in both cell lines in a concentration-related manner. For example, ATP at 0.4 mmolar increased ALP activity in SaOS2 cells by 46%, 40%, and 58% after 2, 5, and 7 days respectively. In MG63 cells the effects on ALP were significantly lower than those seen in SaOS2 cells (0.4 mmolar increased ALP activity by 32%, 39%, and 20% after 2, 5, and 7 days respectively).

The results show that the $P2X_7R$ is expressed by these two osteoblast cell lines and that its expression is modulated by cell density. Furthermore, ATP reduced cell number, but increased ALP activity. The differences in $P2X_7R$ expression levels and the regulation of cell number and ALP activity by ATP are probably related to different stages of differentiation of these cell lines. Overall these findings provide a further insight into the role of the $P2X_7R$ in osteoblast function, and supports the modulation of $P2X_7R$ activity as a new therapy for bone disease.

P49

HSPGS CORE PROTEINS AND GLYCOAMINOGLYCAN SIDE CHAINS ARE ELEVATED IN THE PATHOLOGICAL CONDITION FIBRODYSPLASIA OSSIFICANS PROGRESSIVA.

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Fibrodysplasia Ossificans Progressiva (FOP) is a rare autosomal dominant genetic disorder characterized by congenital malformations of the great toes and progressive heterotopic ossification of the soft connective tissue in characteristic anatomical patterns. FOP is associated with dysregulation of the bone morphogenetic protein (BMP) signaling pathway. Cell surface heparan sulfate proteoglycans (HSPGs) are ubiquitously expressed in almost every cell type; all contain heparan sulfate side chains (glycoaminoglycan (GAG) chains) bound to serine residues of the core protein. Multiple BMPs and their secreted antagonists have HSPG binding domains. We hypothesize that cell surface heparan sulfate proteoglycans (HSPGs) of lymphoblastoid cells play a role in modulating BMP4 signaling through their GAG chains via interactions with BMP ligands and antagonists and that a dysregulation in that process exists in FOP.

Lymphoblastoid cells (LCLs) from control and FOP patients were treated with HSPG core protein specific antibodies, Noggin, and Noggin delta B2 (heparan binding domain removed), BMP antagonists that binds to HSPG GAG chains. Analysis was carried out using FACS analysis, immunofluorescence, and modified ELISA. The presence of glypican 1, syndecan 3, and syndecan 4 was detected on both control and FOP cells with elevated expression on FOP cells. Increased levels of Noggin binding was detected on FOP cell when compared to control cells.

Our results demonstrate that levels of certain HSPGs are elevated in FOP cells when compared to controls. Also, the increase in Noggin binding suggests that GAG chain levels are more abundant in FOP cells. Previous studies using siRNA have demonstrated that HSPGs can have opposing effects on BMP4 signaling, measure by ID1 and ID3 expression, depending on the subtype with a dysregulation seen in enhancing signaling in FOP cells. That effect may be due to elevated levels of HSPG core protein and GAG chains suggested by this study. At present the exact mechanism of increased BMP signaling seen in FOP is unknown. However, our results suggest that the mechanism of dysregulation is influenced and includes the cell surface HSPGs.

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Abstract withdrawn

P51

ESTROGEN RECEPTOR ALPHA MRNA LEVELS ARE HIGHER IN CANINE CORTICAL BONE THAN IN TRABECULAR BONE

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Estrogen (E), acting through estrogen receptor (ER) alpha and ER beta is a major regulator of bone cell function in humans and rodents. These species are polyoestrus which means that their bone cells are exposed to high concentrations of circulating E throughout the year. In contrast, dogs are monoestrus (they only have 1-2 oestrus cycles per annum) and both basal levels of circulating E and peak concentrations at oestrus are 10 times lower than in women. This raises the possibility that either dogs are not dependent on E for maintaining normal bone cell function, or alternatively canine bone cells are extremely sensitive to low levels of E. The aims of the present study were to determine whether ER alpha and ER beta are expressed in canine bone in vivo and to use real time RT-PCR to compare ER alpha mRNA levels in cortical and trabecular bone.

Tibiae from six young, sexually mature dogs were used for mRNA studies (3 male and 3 female). ER alpha mRNA was expressed in cortical and trabecular bone and in bone marrow whereas ER beta mRNA expression in all compartments was extremely low. In each individual ER alpha mRNA expression was higher in cortical than trabecular bone, and when data were pooled these results were statistically significant ($p=0.005$ for trabecular bone, $p=0.02$ for bone marrow). Interestingly, ER alpha mRNA levels were not significantly higher in females than in males. Immunohistochemistry confirmed that ER alpha protein localised in osteoblasts and osteocytes.

In conclusion, these results provide evidence that E, acting through ER alpha, controls bone cell function in monoestrus, as well as in polyoestrus animals. The finding that ER alpha mRNA levels are higher in cortical than trabecular bone, is consistent with the accumulating evidence that ER alpha plays an integral role in mediating bone cells' response to mechanical loading. Our results also suggest that canine bone cells are adapted to respond to low concentrations of circulating E, which would explain why non-traumatic fractures are not a clinical problem in ovariectomised dogs. Establishing the mechanisms involved could have implications for the treatment of osteoporosis in women.

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SUBSTITUTIONS IN THE R1 HYDROXYL AND C1 PHOSPHATE POSITIONS SIGNIFICANTLY AFFECT BINDING OF RISEDRONATE DURING HYDROXYAPATITE CERAMIC CHROMATOGRAPHY

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Bisphosphonates (BPs) bind avidly to bone mineral and their skeletal retention is a significant aspect relevant to their observed biological effects. A hydroxyl (-OH) group in the R1 position together with the presence of the P-C-P group contributes significantly to the noted high affinities. In the present studies we have developed a quantitative and reproducible method to assess relative binding of BPs by using column chromatography employing hydroxyapatite ceramic spheres (20 µm diameter, BioRad) as the column matrix in Fast Performance Liquid Chromatography (FPLC). BPs were eluted from 0.66cm diameter by 6.5cm long glass columns by using a phosphate buffered (pH 6.8) aqueous linear gradient (0.001M-1M) at a flow rate of 2 ml/minute for 50 minutes with elution detection by UV absorbance. Retention times were determined at maximum peak height after loading separately with 0.4 µMoles BP and are shown in the table. The results demonstrate that substitution of R1-OH by -H or halogen groups (F, Cl, or Br) significantly reduces binding affinity. Substitution of one C1 phosphate by carboxyl (NE10790) also strikingly reduces binding. The 2-pyridyl analogue (NE58018) of risedronate (a 3-pyridyl BP) showed significantly higher binding mineral affinity than risedronate and was not significantly different to that seen for zoledronate. The present results confirm that the different affinities of BPs for binding to HAP depend not only on the P-C-P moiety, but also on the R1 and R2 side chains.

BP RETENTION TIMES

Component	Name	R1 substituent	C1 Phosphate	RETENTION TIME (mins)
Ris-OH	Risedronate	-OH	P-C-P	9.97±0.09
Ris-H	NE58043	-H	P-C-P	5.83±0.17*
Ris-Cl		-Cl	P-C-P	6.03±0.03*
Ris-F		-F	P-C-P	5.93±0.07*
Ris-Br		-Br	P-C-P	5.73±0.15*
Ris-COOH	NE10790	-OH	P-C-COOH	4.60±0.06*
Ris-COOH-Cl		-Cl	P-C-COOH	0.00*
Ris-COOH-F		-F	P-C-COOH	0.00*
Ris-COOH-Br		-Br	P-C-COOH	0.00*
NE58018		-OH	P-C-P	12.6±0.03*
NE97221		-H	P-C-P	6.4±0.21*#
Zoledronate		-OH	P-C-P	12.5±0.03*

* Compared with risedronate, p<0.001. # Compared with NE58018, p<0.001

P53

GLUCOCORTICOID METABOLISM IN MURINE BONE CELLS: A MODEL FOR THE EFFECTS OF LOCAL GLUCOCORTICOID GENERATION ON BONE

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Human studies have suggested that local glucocorticoid generation within osteoblasts plays a critical role in bone loss seen during aging, in response to inflammation and treatment with glucocorticoids. Human osteoblasts express the enzyme 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) that converts inactive glucocorticoids (cortisone, dehydrocorticosterone, prednisone) to their active counterparts (cortisol, corticosterone, prednisolone). Enzyme expression increases with age, in response to inflammatory cytokines and with therapeutic glucocorticoids. Reports on glucocorticoid metabolism in mouse osteoblasts have been conflicting with the expression of the glucocorticoid inactivating enzyme 11beta-HSD2 suggested. To establish whether local glucocorticoid metabolism differs between humans and mice we have characterised expression and activity of glucocorticoid metabolising enzymes in bone cells derived from C57BL/6 mice and examined regulation by inflammatory cytokines.

Primary cultures of mouse osteoblasts were derived from calvaria (n=8 mice) and long bones (n=6) of 14-24 week old mice by outgrowth of collagenase treated bone chips. The osteoblastic character of these cells was confirmed by high basal and glucocorticoid-inducible alkaline phosphatase activity (2-fold induction with dexamethasone). 11beta-HSD1 but not 11beta-HSD2 mRNA was detected. Enzyme activity studies revealed predominant glucocorticoid activation (cortisone to cortisol conversion 3.8+/-2.1; dehydrocorticosterone to corticosterone 6.6+/-1.1 pmol/mg/hr) further indicating exclusive 11beta-HSD1 expression. Enzyme activity was similar to that seen in human osteoblasts and did not vary between calvarial and long-bone derived osteoblasts. As in human osteoblasts 11beta-HSD1 expression increased with IL-1beta (8.9+/-2.5 fold mRNA expression, 2.5+/-0.2 fold activity with 10ng/ml IL-1beta). The 11beta-HSD1 cofactor generating enzyme hexose-6-phosphate dehydrogenase (H6PDH) was also expressed and osteoblasts derived from H6PDH knockout mice had a reversal in the predominant direction of enzyme activity towards glucocorticoid inactivation.

Variable expression of 11beta-HSD1 has been reported in human osteoclasts. In mouse marrow monocyte cultures 11beta-HSD1 expression was initially high but fell with treatment with osteoclastogenic factors (m-CSF/RANKL) suggesting an active inhibition of glucocorticoid generation in developing osteoclasts.

These data indicate that glucocorticoid metabolising enzyme expression in C57BL/6 mouse bone cells parallels that in humans and is regulated in a similar fashion. This supports the use of mice as a model for the impact of local glucocorticoid metabolism on bone.

P54

A COMPUTATIONAL MODEL EXPLAINS BISPHOSPHONATE BINDING AFFINITY DIFFERENCES ON HYDROXYAPATITE

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Differences in bone mineral binding affinities of clinically utilized bisphosphonates (BPs) have been described with a decreasing rank order of zoledronate>alendronate>risedronate>etidronate. This may lead to differences in bone uptake and release. A computer-aided 3-D analysis of nitrogen (N) -containing BPs was conducted to explain these differences.

Once a low energy conformation of each BP was oriented in a tridentate binding mode on the trigonal prismatic column of calcium atoms in hydroxyapatite (HAP), the N side chain conformations of the BPs were examined for their interaction with the [001] surface. The 4-amino group of alendronate can form a strong N-H---O hydrogen bond (132°, 2.7Å N---O distance) to the labile -OH oxygen on HAP, where fluoride is known to substitute. The corresponding ring N of zoledronate can only form a weaker electrostatic interaction with this labile -OH site. However it can form an additional strong hydrogen bond to a bifurcated network, between two oxygen coordinated to a calcium in a neighboring column of trigonal prismatic calciums including a 132° angle and a 2.7 Å N---O distance, explaining its high affinity. In the case of risedronate, steric hindrance of the pyridyl ring prevents its N from forming hydrogen bonds in either fashion and it may only form weaker electrostatic interactions, such as at the labile -OH (N---O distance 3.0 Å, 102°) This affords weaker affinity compared to alendronate and zoledronate, but does produce higher affinity than etidronate (no N functional group). Comparative modeling of other BPs further demonstrates this affinity/H-bonding correlation.

There is increasing evidence that the mechanism of action of each BP combines a differing balance of biochemical activity versus bone mineral interactions. This can lead to differing pharmacology and perhaps different bone quality among BPs.

P55

POTASSIUM CHANNEL SUBUNITS IN HUMAN OSTEOBLAST-LIKE CELLS

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Both patch-clamp methods and RT-PCR have shown that MG63 cells possess a number of ion channel subtypes. There is also some evidence that these channels, including the Ca-activated K channel of large conductance (maxi-K), regulate function in these cells. Given the presumed role of K channels in cell proliferation (Chandy et al., 2004), we have now extended our investigations of the characteristics of the maxi-K channel in MG63 cells. We have also investigated the type and possible role of ATP-sensitive potassium channels (KATP) and the associated sulphonylurea receptor (SUR).

RT-PCR was carried out using primer pairs for the alpha and beta1 subunits (KCNMA, KCNMB1 respectively) of the maxi-K channel, Kir6.1 and SUR2B subunits of the KATP channel. Conventional single channel recordings from cell-attached patches were also made. Proliferation assays (up to 120 hours) were carried out using MTS.

Openings of the maxi-K channel can be readily observed in cell-attached patches at resting membrane potential. The scorpion peptide iberiotoxin (IBX), concentration 5.9 nanomolar, blocks the maxi-K channel activity. RT-PCR shows that in addition to the alpha subunit, the beta1 subunit is present.

The presence of the KATP subunit Kir6.1 and SUR2B were also confirmed in MG63 cells using RT-PCR. Multiple channels openings were seen in a high proportion of cell-attached patches at resting membrane potential and hyperpolarised potentials. These channels had a conductance of 46 pS plus or minus 15 pS (n = 4). The open probability showed no voltage sensitivity between resting membrane potential and 100 mV hyperpolarised to rest. These data are consistent with the properties of KATP channels previously reported in a number of cell types. We therefore examined the effects of the KATP channel activator, pinacidil, and the blocker, glibenclamide on cell numbers at both 48 and 120 hours. Interestingly, pinacidil (10-

100 micromolar) significantly increased cell numbers ($p<0.05$), and the blocker glibenclamide (100 micromolar) decreased numbers ($p<0.05$).

Our data continue to support the view that K channels have a role to play in osteoblast-like cell function.

References: Chandy, K. G., et al. (2004). Trends Pharmacol Sci 25(5): 280-9

P56

TRANSCRIPTOMIC ALTERATIONS UNDERPINNING COBALT MEDIATED OSTEOLYSIS

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Background: Improvements in material properties of total joint prostheses and methods of fixation mean that arthroplasty is the most effective means of restoring mobility in patients with osteoarthritis. Aseptic loosening is the major cause of long-term failure of prostheses. Cobalt particles may act directly on osteoblasts, decreasing bone formation and potentially playing a role in osteolysis and aseptic loosening. The aim of this study was to assess gene expression profiles of primary human osteoblasts exposed to cobalt ions in a temporal manner, and to identify the genes and gene clusters underpinning the osteoblast response to cobalt.

Methods: Primary human osteoblasts were exposed to cobalt ions at a concentration of 10ppm. To determine gene expression profiles, comparisons were made between control and 12, 24, 48 and 72 hour time exposures. RNA isolation and cDNA synthesis were performed. Gene profiling of osteoblasts exposed to cobalt ions was carried out using the Affymetrix Human Genome UI33 Plus 2.0 array. Data was normalised using RMA express and an average expression measure for each time point used to identify alterations in gene expression. Validation of data was achieved by performing quantitative real time RT-PCR on selected genes.

Results: Oligonucleotide microarray expression profiling identified significant alterations in osteoblast gene expression in response to cobalt exposure. Distinct phase patterns were observed, with significant altered expression following 12, 24 and 48 hours cobalt ion exposure. Of the 22,233 gene sequences represented on the Affymetrix microarray, 4.8% (1077 genes), 4.1% (930 genes), and 2.13% (486genes) were significantly altered. We identified dysregulation of key functional families in response to cobalt ions, including alterations in cellular proliferation, development and inflammation associated genes. Conclusion: These data will provide novel avenues for exploration in our efforts to further characterize the molecular mechanisms underpinning the initiation and progression of osteolysis.

P57

SYNOVIUM BIOPSIES AND SYNOVIAL FLUID CYTOKINES AT KNEE ARTHROPLASTY

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Osteoarthritis (OA) has been described as a non-inflammatory arthritis although there is evidence that there are aspects of inflammation that are more prevalent in OA synovial joints than normals. NSAIDs are often prescribed to patients awaiting total knee arthroplasty (TKR). However, there is debate as to whether any inflammatory disease elements remain at this point in the progression of the disease, and therefore is the NSAID being prescribed solely as an analgesic.

Synovium biopsy and synovial fluid of 17 patients undergoing TKR were sampled at the onset of their surgery. A 3 point scale was used to assess the cellularity of synovium, a parameter considered to correlate with several markers of OA. A bead array was used to simultaneously quantify the cytokines and chemokines IL-12, TNF, IL-10, IL-6, IL-1, IL-8 in OA synovial fluid.

The 3 point scale used to describe the cellularity of the synovium, placed the majority in groups 2 and 3, the most cellular groups. Low levels (<120 pg/ml) of IL-12, IL-10, IL-1 and TNF were measured in all 3 cellularity groups. Markedly elevated values of IL-6 and IL-8 were measured in the synovial fluid of knees with the most cellular synovium (maximum values were 2721 pg/ml and 840 pg/ml respectively).

We have established that certain inflammatory cytokines are elevated at TKR, and that this is linked to the cellularity of the synovium. We are now investigating whether we can control these aspects of inflammation with CB1 and CB2 cannabinoid receptor agonists in vitro.

P58

LOW DOSES OF HEPARIN STIMULATE RAT OSTEOBLAST DIFFERENTIATION BY POTENTIATING BONE MORPHOGENETIC PROTEIN 2 SIGNALLING

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Sulphated polysaccharides are known to regulate osteoblast differentiation and bone formation and can interact with BMP and may regulate aspects of BMP signalling. This study examines how low doses of heparin (1-100ng/ml) regulate osteoblast differentiation in vitro and investigates the influence of heparin on BMP-2 signalling.

To assess differentiation, primary rat osteoblasts were cultured for 7-28 days with heparin (range 1ng-10microg/ml) in media supplemented with dexamethasone, L-ascorbic acid and beta-glycerophosphate. Alkaline phosphatase activity was determined colourimetrically and bone nodule formation was analysed by Von Kossa staining. Studies of BMP-2 signalling were performed by Western blotting, using phospho-specific antibodies to Smad 1/5/8, Erk and Akt. To follow Smad activity, osteoblasts were transfected with a luciferase reporter construct controlled by Smad-binding elements.

Studying osteoblast differentiation in the presence of heparin demonstrated a significant increase in alkaline phosphatase with low doses of heparin (1-100ng/ml) compared to untreated controls (pvalue less than 0.005). In contrast higher doses of heparin (1-10microg/ml) inhibited alkaline phosphatase activity. Treatment for 28 days with 1ng/ml-10µg/ml heparin showed an increase in bone nodule formation with 100ng/ml only.

Investigating the effects of heparin on BMP signalling demonstrated elevated levels of Smad1/5/8 phosphorylation in samples treated for 20 mins with 10ng/ml heparin and 100ng/ml BMP-2, which were not seen in controls. At longer time points (1, 3 and 5 hours) the combination of BMP-2 and heparin induced a sustained increase of Smad 1/5/8 phosphorylation compared to BMP-2 alone. Using a reporter assay, osteoblasts treated with BMP-2 and heparin showed a three-fold increase in Smad-regulated transcription compared to controls or cells treated with 100ng/ml BMP-2. Investigating PI3 kinase pathway after treatment with 100ng/ml BMP-2 showed a sustained high level of phospho-Akt throughout the time course however, the MAPK pathway showed a decrease in phospho-Erk levels with BMP-2 and BMP-2/heparin treatment after 40 mins.

In conclusion, these data reveal a stimulatory effect of low dose heparin on bone formation and BMP-2 signalling in vitro. We hypothesise that this is mediated by direct interaction with BMP-2 signalling to regulate osteoblast differentiation.

P59

CLONING AND EXPRESSION OF HUMAN BMP-2: POTENTIAL FOR USE IN SKELETAL TISSUE ENGINEERING

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The classical approach to skeletal tissue engineering combines three elements: progenitor/stem cells with osteoblast potential, an osteoconductive scaffold, and osteogenic-specific growth factors. Progress with stromal cells and a whole raft of scaffolds progresses, however, optimisation and provision of growth factor requirements remains elusive. The aim of this study was to clone and express the gene for human bone morphogenetic protein-2 (BMP-2) for utilisation in the development of skeletal functional tissue.

mRNA from Saos-2 cells was used to create a cDNA library. BMP-2 specific primers were used to amplify the BMP-2 gene; this was sub-cloned into a mammalian expression vector with a V5 epitope and

His-tag for purification. Interestingly, the mRNA for BMP-2 was found to be a transcript isoform never before reported in which all of exon 2 has been deleted. The sequence of the mature protein is expressed from the plasmid rather than the pre-propeptide.

The BMP-2 expression plasmid (pcKBMP2) was transiently transfected into CHO cells. After 2 and 5 days, the cells were lysed, the media harvested and concentrated, and these proteins analysed by SDS-Page and Western Blotting. Using the anti-V5 antibody, rBMP-2 was identified in cell lysates and expression of rhBMP-2 confirmed. Activity of rhBMP-2 was assayed using expression of alkaline phosphatase in permissive cells following exposure to BMP-2. Alkaline phosphatase was measured in transiently transfected cells as well as from conditioned media (CM). In the murine chondrocytic cell line, ATDC5, a significant increase in alkaline phosphatase was measured after 2 days compared to cells transfected with a control plasmid. Using the promyoblast cell line, C2C12 cells, to measure alkaline phosphatase activity, CM from CHO cells transfected with pcKBMP-2 was analyzed after 2, 5 or 7 days. Alkaline phosphatase activity was measured only from cultures 7 days after transfection, but not at 2 or 5 days. These results suggest an intracellular accumulation of protein in the initial stages of transfection with eventual secretion by 7 days. Taken together, these results confirm BMP-2 expression and activity and the generation of a recombinant protein and plasmid for optimisation of the requirements for tissue engineering replacement bone.

P60

SOX9 ENHANCES BMP-2-INDUCED OSTEOGENIC DIFFERENTIATION IN A PKA DEPENDENT MANNER

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Sox9, a transcription factor with a high-mobility group DNA-binding domain, plays a critical role in chondrogenesis through activating chondrocyte-specific marker genes, such as Col2a1, Col11a2, and Aggrecan. Inactivation studies of Sox9 before and after mesenchymal condensations suggested that Sox9 may be involved in both chondrogenic and osteogenic cell lineages determination. Previous studies have demonstrated that phosphorylation of Sox9 by PKA (cAMP-dependent protein kinase) can enhance its ability to transactivate chondrocyte-specific enhancer of Col2a1. The current study was to examine the role of Sox9 in regulating osteogenesis and the effects of PKA activity on Sox9 function.

Effect of mouse Sox9 gene overexpression on osteogenesis was investigated using C2C12 cells. C2C12 is a mouse pre-myoblast cell line, when treated with BMP-2, it can acquire osteogenic phenotype expressing Runx2, Col1a1, ALP as well as other osteo-hallmarks. Using Lipofectamine method, a Sox9 mammalian expression plasmid with geneticin-resistant gene was transfected into the C2C12 cells. The stable transfectants (C2C12-Sox9 cells) were selected by culture with geneticin and used for further studies. When treated with rhBMP-2, ALP activity and Col1a1 gene expression were increased in both C2C12-Sox9 cells and C2C12 cells with empty vector, but the ALP activity and Col1a1 gene expression were significantly higher in the C2C12-Sox9 cells. When 8-Br-cAMP, a specific activator of PKA was added to the cells under rhBMP-2 treatment, the ALP activity was further increased in both cell types. When PKA inhibitor-H-89 was added, ALP activity was completely abolished in the two cell types under BMP-2 treatment. ALP activity was reduced when PKIgamma (Protein kinase inhibitor gamma), a predominant isoform of PKIs in C2C12 cells was transfected into the C2C12-Sox9 cells.

Taken together, our data suggest that Sox9 accelerates BMP-2-induced osteogenic differentiation, and it may function in a PKA-dependent pathway. The function of Sox-9 gene and its regulation in osteogenesis and other cell lineage differentiation needs further investigation.

P61

MECHANO GROWTH FACTOR EXPRESSION IN BONE: RESPONSE TO MECHANICAL STIMULATION

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Osteoporotic fracture is a significant cause of morbidity in at risk populations. While mechanical stimulation (exercise) is known to protect against the osteoporotic process the translation of mechanical stimulation into an osteoprotective effect is not fully understood. Mechano Growth Factor (MGF) is an Insulin-like Growth Factor I isoform derived as a consequence of alternative splicing of the IGF-I gene. MGF has been shown to be responsive to high resistance exercise in human muscle however this MGF regulatory system has not been described in bone. Using a 3D bioreactor system we have studied for the first time the cellular and molecular response of human bone to mechanical stimulation.

Trabecular bone cores were prepared from femoral head tissue removed from patients undergoing total hip arthroplasty. Cores were either frozen directly (T0), maintained in the bioreactor system and subjected to mechanical stimulation (3000 µstrain in jumping exercise waveform repeated at 1 Hz for 5 minutes daily) or maintained in the bioreactor under control non-exercise conditions for 7 days. Cryosections were prepared for histochemical analysis of bone formation marker Alkaline Phosphatse. RNA was also extracted from the samples and accurately quantified using Nanodrop 1000 Spectrophotometer and cDNA transcribed using a MGF gene specific primer. Quantitative real time PCR analysis was performed using ABI Prism 7900® system and Applied Biosystems custom TaqMan® MGF gene expression assay.

The percentage Alkaline Phosphatase labelled bone surface was increased in all mechanically stimulated patient samples (n=4) compared to control ($p \leq 0.03$). MGF gene expression was detected in all control patient samples with one patient showing regulation in MGF gene expression in response to mechanical stimulation (Ct value 36.3 ± 0.2 Control vs 35.6 ± 0.4 Mechanical stimulation; R^2= 0.97). This is the first description of MGF expression in bone and increased sample size will be required to determine the true nature of the response to mechanical stimulation however if MGF is shown to induce similar trophic changes in bone as it does in muscle, then it may prove an exciting target for gene therapy or conventional pharmaceutics in the prevention or treatment of osteoporosis and osteopaenia.

P62

CHARACTERISATION OF MESENCHYMAL STEM CELLS ON BESPOKE BIOCERAMIC SCAFFOLDS PRODUCED BY SELECTIVE LASER SINTERING

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Bioactive glass-ceramics are a class of bioceramics that show excellent biocompatibility in vivo and similar mechanical properties to bone. Therefore they are a promising material for orthopaedic tissue engineering applications. Selective laser sintering (SLS) is one of a range of freeform fabrication technologies which is now being used to produce intricate three dimensional scaffolds to match the exact geometry of the graft site of a patient's bone. SLS can be used to process apatite wollastonite (A-W), a bioceramic, into porous patient specific scaffolds using data from computer tomography or magnetic resonance imaging scans. Cultured human mesenchymal stem cells (MSCs) can be made to undergo osteogenic differentiation to mature osteoblasts which initiate the formation of mineralised bone-like deposits. This makes MSCs attractive candidate cells for tissue engineering. We aim to use MSCs to populate porous bioceramic scaffolds produced by SLS to create bespoke biocompatible bone replacement structures.

Optimal seeding densities were determined and MSCs were cultured on A-W bioceramic scaffolds for up to 21 days in medium containing osteogenic supplements. Using scanning electron microscopy we demonstrated that MSCs adhered, spread and remained attached to the surface of the scaffolds for the duration of the culture period. These observations were confirmed by confocal analysis of CellTracker Green-labelled MSCs, actin-stained MSCs and using an

MG63 osteosarcoma line stable transfected with enhanced green fluorescent protein. Fluorescent live/dead analysis demonstrated that the MSCs retained viability on the A-W scaffolds over 21 days in culture. By RT-PCR and immunocytochemistry we showed that expression of type 1 collagen, alkaline phosphatase, osteocalcin and osteonectin increased with time in culture. These data demonstrate the biocompatibility of A-W scaffolds and their potential as patient-specific bone replacement materials.

P63

BONE TISSUE FORMATION FROM HUMAN EMBRYONIC STEM CELLS IN VIVO

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Both embryonic stem cells and adult marrow-derived stem cells hold great promise as cell sources for the assisted repair of musculoskeletal tissues. However a direct comparison of these different cell sources has not been undertaken.

Methods: This study compared the osteogenic differentiation potential of human embryonic stem cells (hESC) with human adult derived stem cells in vivo. hESC lines H7, H9, the mesenchymal-like, telomerised H9 derivative HEF1, the human embryonic kidney epithelial cell line HEK293 (negative control) and adult human mesenchymal stem cells (hMSC), were either used untreated or subject to pre-differentiation protocols treated with osteogenic factors for 4 days prior to injection into diffusion chambers and implanted into the peritoneal cavity of nude mice. The implants were then retrieved after 10 weeks. Histology and gene expression analysis for markers of bone, cartilage and adipose tissue was undertaken.

Results: All hESCs, other than the HEK293 negative control, when pre-differentiated gave rise exclusively to bone in the chambers. In contrast, hESCs that had not been pre-differentiated formed both bone and cartilage in vivo. In contrast, undifferentiated hMSCs did not give rise to bone, cartilage or adipose tissue in vivo while pre-treatment with OS factors engendered both bone and adipose tissues.

Conclusions: These data demonstrate that pre-differentiation protocols used directed the differentiation of the hES cells to exclusively give rise to the cells of osteogenic lineage. The hMSC's were also capable of producing osteogenic cells in vivo but did not demonstrate similar levels of directed differentiation, resulting in cells of the adipose and osteogenic lineage after pre-differentiation.

These findings point to the potential use of hESC derived cells in regenerative medicine applications that require the stable, directed differentiation of cells down the osteogenic lineage.

P64

THE IN VITRO RESPONSE OF HUMAN OSTEOBLASTS AND OSTEOBLAST-LIKE CELLS TO A NOVEL PHOSPHATE BASED SOLUBLE GLASS

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Bone deficit as a result of trauma or surgery often requires bone grafting. Various sources are available to surgeons, autologous grafts, allogenic grafts and synthetic grafts such as hydroxyapatite (HA). Because autologous and allogenic graft materials have limitations in terms of availability and risk of infection, there is a need to explore novel synthetic graft materials. Phosphate based glasses (PBG), have recently been suggested as candidates for this role.

This study compared a range of novel PBG (CaO,Na2O,P2O5, with CaO content 40, 42, 44, 46, 48 mol percent) with HA and tissue culture plastic (TCP) controls, in their ability to support osteogenic proliferation and differentiation in primary human osteoblasts and human fetal osteoblast-like cells in vitro. Scanning electron microscopy, picogreen proliferation assay, lactate dehydrogenase cytotoxicity assay and real-time polymerase chain reaction were used to evaluate cell attachment, proliferation, death and differentiation respectively.

PBG with lower CaO content (40-46 mol percent) significantly inhibited attachment, proliferation and increased the percentage cell death compared to TCP and HA for both cell types ($p<0.01$ for all parameters; Anova). PBG 48 (mol percent) performed significantly better than other PBG across all parameters ($p<0.1$ Anova) but did not match the performance of HA and TCP ($p<0.5$ Anova; all parameters). Across all groups cell death correlated with medium pH (R2=-0.47, P=0.032 Spearmans), which was related to the dissolution rate of each glass [weightloss g/cm2/day] (R2=0.9, P=0.037 Spearmans). PBG <48 mol percent CaO are unlikely to provide suitable osteogenic graft materials because of their effect on pH.

The relationship between PBG type, dissolution rate, medium pH and cell death suggests that inhibition of PBG solubility, (by increasing CaO content) will protect cell proliferation and survival by maintaining physiological pH. The CaO content of these PBG can be easily modified and this encourages further investigations.

P65

PROPORTION OF RED TO YELLOW MARROW DERIVED BY MAGNETIC RESONANCE SPECTROSCOPY, AN INDICATOR OF SKELETAL REMODELLING CAPACITY?

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Bone marrow may be red (haematopoietically active) or yellow (fatty). Red marrow contains osteoclast precursor stem cells, with the rate of bone turnover at skeletal sites perfused with red marrow being higher than those perfused with yellow marrow. The proportions of these two marrow types vary, being dependent upon age and skeletal site. At birth, red marrow is present throughout the skeleton, with conversion from red to yellow marrow occurring first in the extremities. The adult pattern is characterised by the presence of red marrow only in the portions of the axial skeleton and metaphyses of the femora and humeri. Overall there is an approximately 10% decrease in bone marrow cellularity for each decade of life.

We hypothesise that the proportion of red to yellow marrow may provide an indicator of remodelling capacity, with implications for predicting the net resorption associated with osteoporosis and the skeleton's response to treatment.

A pilot study has been undertaken to investigate the feasibility of utilising magnetic resonance spectroscopy, without a contrast agent, to investigate the dependence of the proportion of red to yellow marrow upon age and skeletal site. Using a 3T whole body MRI scanner, three volunteers, a 13 year-old boy, a 28 year-old male and a 57 year-old female were studied. The proportion of water to lipid was recorded, assumed to indicate the proportion of red to yellow marrow, in 2cm cubic volumes of axial L3 lumbar vertebra and peripheral calcaneus. At the axial vertebra, the water to lipid ratios were 2.71, 1.04 and 0.45 for the three subjects respectively. At the peripheral calcaneus however, the ratio was approximately 0.05 for all three subjects.

As predicted, the proportion of red to yellow marrow at the axial skeleton decreased with increasing age, with the peripheral skeleton being predominantly yellow by the second decade. In conclusion, we have demonstrated the feasibility of non-contrast magnetic resonance spectroscopy to quantify the proportion of red to yellow marrow at axial and peripheral skeletal sites.

P66

OSTEOGENIC DIFFERENTIATION OF INFRAPATELLAR FAT PAD DERIVED STEM CELLS AND THEIR POTENTIAL IN CLINICAL APPLICATIONS

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Infrapatellar fat pad derived stem cells present a potentially viable source of stem cells for the repair of cartilage, bone and for other tissue engineering applications. Monolayer culture of stem cells with beta-glycerophosphate supplemented medium has been shown to induce osteogenic differentiation in bone marrow derived stem cells. We explore the hypothesis that this effect would also apply to stem cells derived from the infrapatellar fat pad.

Infrapatellar fat pad tissue was obtained from patients undergoing total knee replacement surgery (n=3). The tissue was digested and infrapatellar fat pad derived progenitor cells were isolated and

expanded in monolayer culture. The cells were expanded up to passage 2 and were then placed either in osteogenic medium or in control medium for 21 days. Osteogenic medium contained beta-glycerophosphate, dexamethasone and L-ascorbic acid-2-phosphate. Gene expression analysis for alkaline phosphatase and osteocalcin, and von kossa and alizarin red staining for calcification were performed on the cells to assess osteogenesis.

Cells grown in osteogenic medium showed improved osteogenesis as determined by relatively higher gene expression of alkaline phosphatase and osteocalcin genes compared to cells cultured in control medium ($p<0.05$). Von kossa and alizarin red staining confirmed enhanced production of calcium and phosphate by the cells cultured in osteogenic medium.

Our findings show that cells derived from the infrapatellar fat pad have osteogenic potential. Cells expanded in control medium failed to show osteogenic differentiation suggesting that the osteogenic effect is due to beta-glycerphosphate supplemented medium. These findings have important implications for future tissue engineering applications of these cells.

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A 3D CULTURE SYSTEM TO INVESTIGATE MECHANICALLY-STIMULATED GLUTAMATE AND ADENOSINE SIGNALLING PATHWAYS IN OSTEOCYTES

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The molecular basis of memory is not well understood although altered responses at synapses, such as Long Term Potentiation (LTP), are associated with memory and learning. LTP can be explained by modification in the activity of glutamate receptors or via inhibition of these receptors with adenosine receptors. Since glutamate receptors and transporters are mechanically regulated in bone in vivo, we have investigated whether glutamate and adenosine signalling pathways interact in cells of the osteoblast lineage to determine whether such pathways contribute to 'strain memory' in bone.

Osteoblasts express functional ionotropic and metabotropic glutamate receptors, and release glutamate upon receptor activation. We have detected mRNA expression for all adenosine receptors (A1, A2a, A2b and A3) and the enzymes involved in the catabolism and metabolism of adenosine (CD73 and adenosine deaminase) in osteoblast-like (HCC1, MG-63, SaOS-2) and osteocyte-like (MLO-Y4) cell lines. Reverse-phase HPLC revealed that MLO-Y4 cells cultured in monolayers on type I collagen constitutively produce relatively little adenosine (0.1_M/106cells/hr) compared with osteoblasts (5-7_M/106cells/hr). Adenosine production by MG63 cells was stimulated in the presence of 0.01 and 0.1 mM glutamate, by 10 and 15 fold respectively.

To investigate osteocyte responses to mechanical stimulation we have devised a 3D culture system. MLO-Y4 cells were cultured in type I collagen gels (2mg/ml) at a density of 2 X 106 cells/ml. The osteocytes retained their dendritic morphology for at least 72hrs and expressed osteocalcin and GLAST-1.

MLO-Y4 osteocytes in collagen gels were mechanically loaded after depletion of nucleosides and glutamic acid in media. Compressive loads (5% deformation, 3Hz) were applied either according to an osteogenic, intermittent loading regime (90 cycles, 3 hours rest, 90 cycles) or a desensitising regime (180 cycles). Cells and media were analysed after loading to determine release of glutamate, adenosine and ATP and relate this to glutamate and adenosine receptor trafficking, and cell-cell interactions via gap junctions.

We are optimising this culture system to determine whether glutamatergic and adenosine signalling is activated by mechanical loading of osteocytes and self-modifies according to the type of force applied. This may start to provide a molecular basis for strain memory in osteocytes.

Speaker Profiles

Mike Adams

Mike Adams graduated in Natural Philosophy (Physics) from Edinburgh University in 1975, and followed this with a PhD in spinal mechanics from the University of Westminster. His publications mostly concern the mechanical properties of the spine, intervertebral disc (dys)function, and the mechanics of articular cartilage. In 2002 he published the book "Biomechanics of Back Pain" with Nik Bogduk, Kim Burton, and Trish Dolan. He is currently Senior Research Fellow in the Department of Anatomy at the University of Bristol.

Mike's latest research concerns how degenerated intervertebral discs influence their adjacent vertebrae, and how injecting cement into the latter can help matters. However, the subject of his presentation in Southampton arises from years of teaching musculoskeletal biology to undergraduates. Students often ask obvious questions which the experts ignore, perhaps because they are difficult or controversial. “What is disc degeneration?” is one such question, and he intends to tackle it head-on!

Frazer Anderson

Senior Lecturer in Geriatric Medicine at the University of Southampton, Dr Anderson graduated from Edinburgh University in 1986 and undertook training posts in Durham and Northumberland. He developed an interest in osteoporosis while working for Dr Roger Francis in Newcastle-upon-Tyne. He moved to his current post in 1995 and has spent ten years working on large clinical trials of vitamin D and calcium supplementation for fracture prevention in older people.

Nick Bishop

Nick Bishop has been Professor of Paediatric Bone Disease in Sheffield since 1998, having trained in Manchester and Cambridge UK, and Montreal, Canada. His main research interests are treatment strategies in osteogenesis imperfecta, other causes of osteoporosis and recurrent fractures in children, and osteoclast formation defects leading to osteoclast-poor osteopetrosis.

Lynda Bonewald

Dr Lynda F Bonewald is a Curators’ Professor and the Lee M. and William Lefkowitz Professor in the Department of Oral Biology and directs the Bone Biology Research Program (http://dentistry.umkc.edu/oralbiology/bonewald_boneprog.htm). Dr. Bonewald has worked in the area of transforming growth factor beta and in the lipoxygenases but is probably best known for her research in osteocyte biology. Osteocytes are the most abundant cell in bone and may be responsible for sensing mechanical stress and signaling bone modeling and remodeling. She is Director of a program project entitled “The Effects of Mechanical Strain on Osteocyte Function”. The program involves investigators at UMKC, KUMC, and UTHSC in San Antonio, Texas. This program project is composed of four projects and four cores to investigate the effects of mechanical strain on osteocyte function. Dr. Bonewald is presently the Chair of the Advocacy Committee for ASBMR. She previously chaired the Board of Scientific Counselors of the NIH’s National Institute of Dental and Craniofacial Research and is Past President of the Association for Biomolecular Resource Facilities. She has also been a member of the Board of Directors and the Public Affairs Executive Committee of the Federation of American Societies for Experimental Biology. She serves on the editorial boards for the Journal for Biomolecular Techniques and now the Journal for Bone and Mineral Research and is Associate Editor for Experimental Biology and Medicine.

Cyrus Cooper

Cyrus Cooper is Professor of Rheumatology and Director of the MRC Epidemiology Resource Centre at the University of Southampton Medical School and Southampton General Hospital in the UK. Professor Cooper graduated from the University of Cambridge and St Bartholomew’s Hospital, London in 1980, and completed his residency in 1985 at the Southampton University Hospitals. He then worked in the MRC Environmental Epidemiology Unit as an MRC Training Fellow, and at the University of Bristol as a Senior Registrar in Rheumatology. In 1990, he won an MRC Travelling Fellowship to the Mayo Clinic, USA, where he continued his research in osteoporosis. Cyrus Cooper returned to the UK in 1992 to take up a position as Senior Lecturer in Rheumatology and MRC Senior Clinical Scientist. He was promoted to the foundation Chair in Rheumatology at the University of Southampton in 1997 while continuing as an MRC Senior Clinical Scientist at the MRC Environmental Epidemiology Unit. In 2003, he was appointed Director of the MRC Epidemiology Resource Centre, University of Southampton.

Richard Eastell

Professor Eastell is Professor of Bone Metabolism at the University of Sheffield where he is also Deputy Director of the Division of Clinical Sciences (North). He is an Honorary Consultant Physician in metabolic bone disease at the Northern General Hospital, Sheffield. He qualified in medicine from Edinburgh in

1977. He trained at the Mayo Clinic under Dr B L Riggs for 5 years. He became a fellow of the Royal College of Physicians of London in 1996, an honorary fellow of the Royal College of Physicians of Ireland in 1998 and a Fellow of the Royal College of Physicians of Edinburgh, the Royal College of Pathology and the Academy of Medical Sciences in 2000.

He is the head of the Academic Unit of Bone Metabolism Group and has an active research group into the pathophysiology, diagnosis and treatment of osteoporosis. He has published over 200 papers on osteoporosis and related topics. In 1997, he was awarded Hospital Doctor of the Year in the osteoporosis category, in 1998 he was awarded the Corrigan Medal of the Royal College of Physicians of Ireland, and in 2003, was part of the team awarded the Queen's Anniversary Award to the University of Sheffield for the Health and Social Care of Older People. In 2004, he was awarded the Kohn Foundation award from the National Osteoporosis Society and the Society of Endocrinology Medal. He is on the editorial board of Osteoporosis International, Osteoporosis Review, and Journal of Clinical Endocrinology and Metabolism. He is the President of the UK Bone Research Society and the President of the European Calcified Tissue Society. He is Deputy Chairman of the Scientific Advisory Group of the National Osteoporosis Society. He is a member of the MRC Physiological Medicine and Infections Board.

Alicia J El Haj

Alicia J El Haj received an MSc from Manchester and a PhD from Aberdeen. Following two postdoctoral positions in Medical and Veterinary Schools in Belfast and London, she took up a lectureship at Birmingham in 1989. In 1997, she moved to Keele University to develop and expand research in cell engineering as part of a new joint Medical School between Manchester and Keele. Appointed to Research Director, she was instrumental in forming and expanding the Institute for Science and Technology in Medicine. The Institute, based on campus, at the UHNS and the RJAH Orthopaedic Hospital has been rated 5A - 5* in the past 3 RAEs. The Institute research programme is at the clinical interface, with ACI ongoing, and more stem cell treatments planned in orthopaedic repair. Her research is in the field of bone cell transduction of physical processes and translating this research into connective tissue engineering and regenerative medicine funded by the BBSRC, EPSRC, Welcome and EU with extensive publications in the field. She is President of the UK Cell and Tissue Engineering Society and Member of the IFMBE Working Group for Cellular Engineering.

Christopher Evans

Christopher Evans holds the Robert Lovett Chair of Orthopaedic Surgery at Harvard Medical School where he is Director of the Center for Molecular Orthopaedics. He obtained a B.Sc. in Genetics and Microbiology, and a Ph.D. in Biochemistry from the University of Wales. After a period of post-doctoral research in the Department of Molecular Biology, Universite Libre de Bruxelles, Belgium, he came to the University of Pittsburgh Medical School ending up as the Henry Mankin Professor of Orthopaedic Surgery and Professor, Department of Molecular Genetics and Biochemistry. While at the University of Pittsburgh he obtained a MA in the History and Philosophy of Science.

Dr Evans's research interests focus on the application of gene therapy to treat disorders of bones and joints, a field he helped to found.

Dr Evans is immediate Past-President of the Orthopaedic Research Society and a Fellow of both the Royal Society of Chemistry and the Royal College of Pathologists.

John Fisher

Professor John Fisher is Deputy Vice Chancellor at the University of Leeds, Professor of Mechanical Engineering and Director of the Institute for Medical and Biological Engineering. The Institute has over 80 Post Doctoral Researchers and Doctoral research students, working in the field of tissue replacement and tissue engineering, and is supported by a current external research income of over £10 million.

Professor Fisher has over 25 years experience in Medical Engineering research, and has published over 350 journal papers in this field. In the last 10 years his research has focused on wear, wear debris of artificial joints. His laboratory has over 100 functional joint simulation systems making it one of the largest research facilities worldwide. More recently novel coupled biomechanical wear and biological cell culture systems have been developed for pre clinical assessment of the functional osteolytic potential of wear debris and joint replacements. Current research also focuses on cartilage tissue substitution, spine biomechanics and tissue engineering. The work is supported by major collaborative programme funding from EPSRC (Portfolio), NIH, EU and Industry with key international collaborations in USA, Europe, China and Japan.

Mike Horton

Unfortunately Mike is unable to attend the meeting due to long-term illness. We wish him well, and in the meantime welcome his colleague Laurent Bozec who has kindly agreed to give his talk.

John Kanis

Professor Kanis is director of the WHO Collaborating Centre for Metabolic Bone Diseases at Sheffield. His interest in bone disease covers basic, clinical research, health technology assessment, epidemiology and health economics. He is an author of more than 500 scientific publications and a Royal Society of Medicine Award Winner for his Textbook on Osteoporosis. He is an Editor of Bone and sits on the Editorial Board of several peer review journals. He is a long-standing advisor for Government Departments and non-Governmental organisations in several countries. He founded the International Osteoporosis Foundation in 1988 and was Chairman of its Scientific Advisory Board until 1998. He coordinates and participates in many international research collaborations and has served on the project management team of several EC-funded research awards (eg EVOS, EPOS).

Richard Keen

Richard Keen graduated from St Mary's Hospital, London, UK. He is now Director of the Metabolic Bone Disease Unit at the Royal National Orthopaedic Hospital, Stanmore. Dr Keen also holds a senior lecturer appointment in rheumatology and metabolic bone diseases at the Institute of Orthopaedics and Musculoskeletal Sciences, University College London.

Dr Keen heads a clinical research team, and is currently investigating potential new treatments for osteoporosis and other bone disorders. He is the chief investigator for the ZODIAC Study, which is assessing the role for bisphosphonates in the management of bone loss following spinal cord injury. He heads the VIDEO Study, an arc funded clinical trial examining the effect of vitamin D supplementation on symptomatic knee osteoarthritis. Dr Keen also works with the media and is often quoted on medical matters relating to osteoporosis and arthritis.

Outside of medicine, Richard is married with three children. He is a keen sportsman, with his loves being cricket and rugby. He is a qualified mini/youth rugby coach, and his winter weekends are often spent running around on a wet pitch somewhere in northwest London.

John Loughlin

Dr John Loughlin completed his PhD in developmental biology at Leeds University in 1991, before commencing his postdoctoral research in the molecular genetic analysis of monogenic diseases of the extracellular matrix at Oxford University. His postdoctoral supervisor was Professor Bryan Sykes. In 1995 Dr Loughlin went on to establish his own group and to research the genetic basis of primary osteoarthritis (OA) at the Wellcome Trust Centre for Human Genetics, which was situated on the Nuffield Orthopaedic Centre site in Oxford. Publishing the first ever OA genome scan in 1999, Dr Loughlin and his colleagues now focus on an analysis of genes within refined regions of the genome.

Dr. Loughlin's continuing research aims to identify and understand the causal mutations that encode for OA susceptibility. In 1997 Dr Loughlin secured a Postdoctoral Research Fellowship from the Arthritis Research Campaign and in 2002 became a University Lecturer at Oxford. Dr Loughlin teaches medical genetics and supervises postgraduate and postdoctoral research scientists. He is an editorial board member of the Journal Musculoskeletal Sciences and he has given oral presentations of his group's research at workshops, conferences and at Universities in Asia, Europe and North America.

Dr Loughlin has published widely in a number of journals including Nature Genetics, PNAS, BMJ and the American Journal of Human Genetics.

Stephen Minger

Dr Stephen Minger is the Director of the Stem Cell Biology Laboratory and a Senior Lecturer in the new Wolfson Centre for Age Related Diseases at King's College London. Dr Minger received his PhD in Pathology (Neurosciences) in 1992 from the Albert Einstein College of Medicine. From 1992-1994, he was a post-doctoral fellow at the University of California, San Diego, where he first began to pursue research in neural stem cell biology. In 1995, Dr Minger was appointed an Assistant Professor in Neurology at The University of Kentucky Medical School. He moved his stem cell research programme to Guy's Hospital in 1996 and was appointed a Lecturer in Biomolecular Sciences at King's College London in 1998. Over the last 15 years, his research group has worked with a wide range of somatic stem cell populations, as well as mouse and human embryonic stem (ES) cells. In 2002, together with Dr Susan Pickering and Professor Peter Braude, Dr Minger was awarded one of the first two licenses granted by the UK Human Fertilisation and Embryology Authority for the derivation of human ES cells. His group subsequently generated the first human embryonic stem cell line in the UK and was one of the first groups to deposit this into the UK Stem Cell Bank. They have gone on to generate three new human ES cell lines, including one that encodes the most common genetic mutation resulting in Cystic Fibrosis.

In addition to the derivation of human ES cell lines, the Stem Cell Biology Laboratory is focused on the generation of a number of therapeutically relevant human somatic stem cell populations from embryonic stem cells. These include cardiac, vascular, retinal, and neural stem/progenitor cell populations, as well pancreatic ß-cells and oligodendrocyte progenitors. Dr Minger has established highly productive collaborations with a number of specialist groups in many areas of clinical interest throughout the UK, and is one of the co-organisers of the London Regenerative Medicine Network, a grass-roots, research-led organisation designed to stimulate clinical translation of cell- and gene-based therapies within London. He is also the Senior Editor of Regenerative Medicine, a new journal launched in Jan 2006 by Future Medicines.

Dr Minger's research is supported by the UK Medical Research Council, The European Union, The Oliver Bird Foundation, The Wellcome Trust, The UK Department of Trade and Industry, The Charitable Foundation of Guy's and St Thomas' Hospitals, the BBSRC, and the EPSRC amongst others.

John Newell-Price

Dr John Newell-Price graduated in Medicine from Cambridge University in 1990. He underwent training in Endocrinology at St Bartholomew's Hospital: from 1993 as a Lecturer, and was an MRC Training Fellow from 1995-8 whilst doing his PhD. Since April 2000 he has been Senior Lecturer in Endocrinology at University of Sheffield and Sheffield Teaching Hospital Trust, and was elected FRCP in 2004. His specialist clinical interest is in pituitary disease, Cushing's syndrome and neuroendocrine tumours, and basic science interest is in the regulation of hormonal gene expression, and in particular epigenetic mechanisms involved in gene silencing.

Ian Reid

Ian Reid MD is Professor of Medicine and Endocrinology at the University of Auckland, New Zealand. His research interests include the pathogenesis and management of osteoporosis, primary hyperparathyroidism & Paget's disease, and his research group has been active in the identification of novel regulators of bone cell function. He is President of the International Bone and Mineral Society, Secretary of the Asian Pacific Osteoporosis Foundation, and a Fellow of the Royal Society of New Zealand.

Jonathan Tobias

Jonathan Tobias is a Reader in rheumatology at University of Bristol, and consultant rheumatologist at Bristol Royal Infirmary, where he has been based since 1995. His undergraduate studies in medicine were at Cambridge University and London University from where he qualified in 1984, followed by MD and PhD theses in bone biology which he completed in 1990 and 1994 respectively, at St George's Hospital Medical School, London. His research, which has led to 49 original research papers and 18 book chapters and reviews, focuses on the regulation of osteoblast activity by estrogen receptors and other anabolic factors, the management of osteoporosis in adults, and the factors which influence bone development in childhood. He has extensive clinical experience in treating patients with osteoporosis, and in running DXA-based osteoporosis diagnostic services. He serves on the editorial board of Calcified Tissue International, the scientific program committees of the American Society for Bone and Mineral Research, the British Society for Rheumatology and National Osteoporosis Society, and on the research committee of the Arthritis Research Campaign. He is also treasurer of the Bone Research Society.

Marian Young

Marian F. Young is Chief of the Molecular Biology of Bones and Teeth Section in the Craniofacial and Skeletal Diseases Branch of the National Institute of Dental and Craniofacial Research. She received her BS from SUNY at Oneonta, NY (1976), and her PhD in Developmental Biology from the Department of Genetics and Cell Biology at the University of Connecticut (1981). After a fellowship in the Lab of Developmental Biology and Anomalies at the NIDR (1981-1983) Dr. Young headed a group in the Mineralized Tissue Research Branch also at the NIDR where she began her investigations on the molecular biology and function of ECM proteins in skeletal tissues. Her current research focuses on regulation and function of small proteoglycans in mineralized tissues and in their potential role in controlling pathological skeletal conditions such as osteoporosis, osteoarthritis and ectopic ossification. Dr. Young has published over 100 peer-reviewed articles and numerous reviews and book chapters on the molecular biology of ECM in mineralizing tissue.

Exhibitor Profiles

Eli Lilly and Company

Eli Lilly and Company 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 therapeutic areas.

To assist in the therapeutic management of Osteoporosis, Lilly has two products, each catering for different patient needs.

For information about either of our osteoporosis products or services, please come and talk to us at the Lilly stand. We look forward to meeting you.

Immunodiagnostic Systems

Immunodiagnostic Systems (IDS) offers a range of esoteric immunoassay kits for clinical and research use. The company focuses in Bone & Mineral Metabolism and Growth Factor research, and engages in collaborative projects with researchers worldwide.

IDS is a leader in the field of Vitamin D analysis, offering both manual and automated 25(OH) Vitamin D methods, an award-winning 1,25-Dihydroxy Vitamin D RIA system, and now announces OCTEIA 1,25(OH)2 Vitamin D, an EIA employing the proven immunocapsule sample preparation.

IDS offers a full range of Bone & Skeletal products, including BoneTRAP® (Tartrate-Resistant Acid Phosphatase 5b), MouseTRAP™, RatTRAP™, Bone-Specific Alkaline Phosphatase (Ostase® BAP), Intact PTH, urinary DPD, RANKL & OPG for both clinical and research (animal) use.

IDS OsteoSite™ antibodies include multiple forms of PTHrp, Cathepsin K, Osteoarthritis Matrix Antigen (a Collagen VI epitope) and a specific Osteoclast Antigen; the GroPep range has multi-species Growth Factor and GF-Binding Protein antibodies; and the Eurodiagnostica range has antibodies to many matrix antigens, neuropeptides and associated reagents.

Dentine discs are available for osteoclast activity studies in vitro, suitable for use in microtitre wells.

Merck Sharp & Dohme Limited

Merck Sharp & Dohme Limited (MSD) is the UK subsidiary of Merck & Co., Inc. of Whitehouse Station, New Jersey, USA, a leading research-based pharmaceutical company that discovers, develops, manufactures and markets a wide range of innovative pharmaceutical products to improve human health.

MXD

MXD Ltd. are distributors for OsteoGram Osteoporosis software. A standard 8 by 10 inch hand X-Ray is used. Two posterior anterior of left hand fingers are acquired at two differing intensity, using a template with an aluminium wedge phantom. From this BMD is obtained. Reference is made to a US data base and T and Z scores are obtained.

MXD Ltd will also show the DicoMeasure orthopaedic measurement software for pre and post operative analysis and review of implants.

For information about either the Osteoporosis or Orthopaedic software, please come and talk to us at the MXD stand. We look forward to meeting you.

Olympus KeyMed

A wholly-owned subsidiary of the Olympus Corporation of Japan, Olympus KeyMed is an important part of the global Olympus network, with specific responsibility for the development and manufacture of medical and industrial equipment for distribution worldwide. Olympus KeyMed's medical business is made up of six distinct divisions, giving a complete focus to all aspects of the healthcare market. The Imaging Systems division is responsible for the Olympus range of arthroscopy products, and the popular TrueView range, including the innovative TrueView Direct Integrated Camera System, which will be on show at the Bone Research Society and British Orthopaedic Research Society First Joint Meeting.

For further information, visit the company's website at www.keymed.co.uk

Orthodynamics

Orthodynamics has expertise as a developer, manufacturer and supplier of specialist hip and knee implants for use in orthopaedics. The exciting product portfolio has an enviable reputation of being able to provide the orthopaedic surgeon with both off the shelf and custom made hip and knee implants for specific patients' needs. Established by Dr. John Bradley in 1986, and now part of the Summit Medical Group since 2003, the company has grown to command a high level of clinical respect and regard within the United Kingdom, and in key overseas markets.

For further information, visit our website, www.orthodynamics.co.uk

Oxford Biosystems Ltd

Oxford Biosystems is a supplier of in-vitro-diagnostics and immunochemicals for routine and research. We

are specialized in new innovative immunoassays and other analytical methods e.g. HPLC, PCR, in different areas of medical laboratory diagnostics, medical research and industrial laboratories. All our products are sourced from innovative organizations that have a proven track record in developing and manufacturing diagnostic kits.

Oxford Biosystems has become a leading resource for clinical researchers looking for high-performance diagnostic kits in a number of areas:

Androgen Assessment, Anemia, Angiogenic Factors, Animal Testing, Anti-Ageing, Preventive Medicine, Autoimmune, Biogenic Amines, Bone & Mineral Metabolism, Cardiovascular, Renal Function, Cytokines, Diabetes Related, Eicosanoids, Energy Metabolism, Body Weight Reduction, Fertility, Reproduction, Gastroenterology, Nutrition, Growth Markers, Infection, Inflammation, Immunology, Mineral/Salt, Neural Tissue Damage, Oncology, Oxidative Stress, Thyroid Function, Research antibodies.

Pfizer

Pfizer, with its UK business headquarters in Surrey and global headquarters in New York, is a research-based global pharmaceutical company. Pfizer discovers, develops, manufactures and markets leading prescription medicines for humans and animals, and many of the world's best-known consumer treatments. Every month, over two million patients in the UK are prescribed a Pfizer medicine. It is estimated that on any given day, 40 million people around the world are treated with a Pfizer medicine.

PromoCell

PromoCell provide a complete cell culture system with a multitude of human cells. In addition to the cells, each cell culture system contains the special growth medium optimized by PromoCell and which is ready to use. The media are either serum-free or contain as little serum as possible. The solutions necessary for the sub-cultivation of human cells are also supplied by PromoCell.

You will find Cytokines, ELISA Kits and Antibodies as well as further interesting products for the cell biology field (e.g. Reagents and Kits for cell transfection and cell analysis) in our PromoKine catalogue which will be published soon.

Regenerative Medicine

Regenerative Medicine provides a novel forum to address the important challenges and advances in the exploding field of stem cell research and regenerative medicine. Senior Editor, Stephen Minger (Kings College London, UK) and a panel of more than 60 international experts ensure that Regenerative Medicine delivers high quality, topical reviews, primary research, editorials and technology evaluations, all subject to rigorous peer review.

Roche and GlaxoSmithKline

Roche is one of the world's leading research-based healthcare groups. With the aim of enhancing people's health and quality of life, our core businesses in pharmaceuticals and diagnostics provide innovative products and services for the prevention, diagnosis and treatment of disease. In the UK, Roche employs around 1,800 people in prescription and over the counter medicines and diagnostics. Together, our businesses in the UK strive to make a real difference to healthcare, the UK's health services and, most importantly, people's lives.

GSK, one of the world's leading research-based pharmaceutical and healthcare companies, is committed to improving the quality of human life by enabling people to do more, feel better and live longer.

Servier Laboratories

Servier Laboratories is the UK subsidiary of The Servier Research Group, the leading independent French research based pharmaceutical company established in 1954.

The key franchises of the Servier Research Group are:

- Cardiovascular disease
- Diabetes
- Rheumatology
- Central Nervous System
- Oncology

In 2005, Servier Laboratories had an annual UK turnover of close to £100 million and now ranks within the top 15 largest pharmaceutical companies in the UK.

Servier Laboratories was the fastest growing pharmaceutical company in the UK in 2005 with a growth rate of 29% against an industry average of around 6%.

Shire Pharmaceuticals Group plc

Shire Pharmaceuticals Group plc (Shire) is a global specialty pharmaceutical company with a strategic focus on meeting the needs of the specialist physician and currently focuses on developing projects and marketing products in the areas of central nervous system (CNS), gastrointestinal (GI), and renal diseases. Shire has operations in the world's key pharmaceutical markets (US, Canada, UK, France,

Italy, Spain and Germany) as well as a specialist drug delivery unit in the US.

For further information on Shire, please visit the Company's website: www.shire.com

Stryker

Stryker Corporation is a leader in the worldwide orthopaedic market and is one of the world's largest medical device companies. Stryker delivers results through a wide range of capabilities including joint replacements, trauma, spine and micro implant systems, orthobiologics, powered surgical instruments, surgical navigation systems, endoscopic products as well as patient handling and emergency medical equipment.

We've invested over 20 years of research in developing orthobiologic technologies. These regenerative products can help patients who previously had few, if any, treatment options. This research also holds the promise of creating other kinds of orthobiologic regeneration -- with the potential to provide better results for countless people.

The Alliance for Better Bone Health

The Alliance for Better Bone Health was formed by Procter & Gamble and Aventis part of the sanofi-aventis Group, in May 1997 to promote bone health and disease awareness through numerous activities to support physicians and patients around the globe.

Procter & Gamble

Three billion times a day, P&G brands touch the lives of people around the world. The company has one of the strongest portfolios of trusted, quality, leadership brands, including Actonel®, Asacol®, Vicks®, Pepto-bismol®, Metamucil®, Thermacare®, Crest®, Oral-B®, Pampers®, Ariel®, Always®, Pantene®, Herbal Essences®, Mach3®, Fairy®, Ace®, Lenor®, M. Propre®, Tampax®, Tempo®, Dash®, Pringles®, Iams®, Eukanuba®, Duracell®, Olay®, Head & Shoulders®, Wella, Gillette®, and Braun.

The P&G community consists of almost 140,000 employees working in over 80 countries worldwide. Please visit http://www.pg.com for the latest news and in-depth information about P&G and its brands. For more information about P&G Pharmaceuticals, please visit www.pgpharma.com.

sanofi aventis

Sanofi-aventis is the world's third-largest pharmaceutical company, ranking number one in Europe. Backed by a world-class R&D organization, sanofi-aventis is developing leading positions in seven major therapeutic areas: cardiovascular, thrombosis, oncology, metabolic diseases, central nervous system, internal medicine, and vaccines. At sanofi-aventis we are committed to researching, developing and bringing to market new and innovative healthcare products so we can fulfil our mission… Because health matters.

Technoclone

Technoclone 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 Metra® range of Bone Marker assay kits from Quidel and the Bone and Cartilage assay kits from Nordic Bioscience Diagnostics.

The Nordic Bioscience range includes clinical tests for serum and urine CTX-I (CrossLaps®),N-MID Osteocalcin and urine CTX-II (CartiLaps®), as well as the preclinical RatLaps and CrossLaps for Culture CTX-I kits, Rat-MID Osteocalcin and serum and urine pre-clinical CartiLaps® (CTX-II) kits. Nordic Bioscience also supply bovine bone slices available in a range of sizes for different assay plates.

The Metra® 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, alpha-1 Helical Peptide and YKL-40, as well as a chromogenic Creatinine assay and other research reagents.

Details of new kits from both Nordic Bioscience and Quidel will be available at the BRS meeting including

- Metra® TRAcP 5b ELISA
- Alpha CrossLaps (CTX-I) for bone metastases
- Calcitonin ELISA
- Intact PTH ELISA
- Metra® Osteoprotegerin

Technoclone also markets the IBEX range of Cartilage diagnostics and an extensive range of autoimmune assays, complement diagnostics and multiplex cytokine assays.

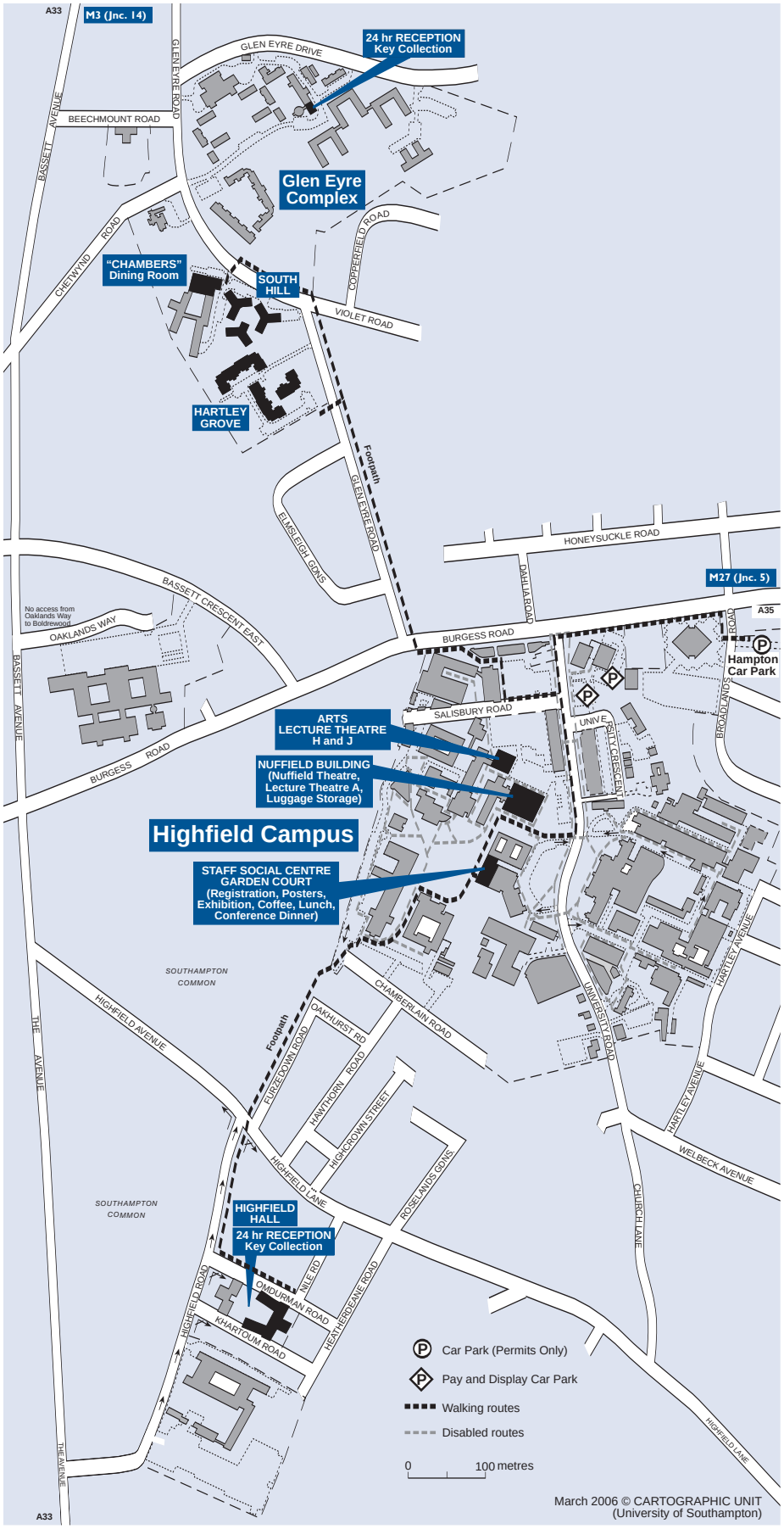
Zimmer Ltd

Zimmer is the world's #1 pure play orthopaedic company, so when it comes to keeping up with what's new in orthopaedics a visit to our stand is essential.

Zimmer is leading the way in Minimally Invasive Surgery (MIS™), new applications such as Trabecular Metal™ Technology providing innovative solutions for cementless fixation, and Durom™ Hip Resurfacing drawing upon the experience of over 250,000 metal-on-metal (Metasul) implantations.

Our specialist staff will be available throughout the exhibition and will be delighted to update you on all Zimmer's latest products and technologies.

Map


A33
M3 (Jnc. 14)
GLEN EYRE DRIVE
24 hr RECEPTION
Key Collection
GLEN EYRE ROAD
BEECHMOUNT ROAD
BASSETT AVENUE
Glen Eyre Complex
CHETWYND ROAD
"CHAMBERS" Dining Room
SOUTH HILL
COPPERFIELD ROAD
VIOLET ROAD
HARTLEY GROVE
Footpath
GLEN EYRE ROAD
ELMSLEIGH GDNS
HONEYSUCKLE ROAD
DAHLIA ROAD
M27 (Jnc. 5)
A35
No access from Oaklands Way to Boldrewood
OAKLANDS WAY
BASSETT CRESCENT EAST
BURGESS ROAD
BROADLANDS ROAD
Hampton Car Park
SALISBURY ROAD
UNIVERSITY CRESCENT
ARTS LECTURE THEATRE H and J
NUFFIELD BUILDING (Nuffield Theatre, Lecture Theatre A, Luggage Storage)
Highfield Campus
STAFF SOCIAL CENTRE GARDEN COURT (Registration, Posters, Exhibition, Coffee, Lunch, Conference Dinner)
SOUTHAMPTON COMMON
HARTLEY AVENUE
HIGHFIELD AVENUE
THE AVENUE
Footpath
OAKHURST RD
CHAMBERLAIN ROAD
UNIVERSITY ROAD
FURZEDOWN ROAD
HAWTHORN ROAD
HIGHCROWN STREET
WELBECK AVENUE
CHURCH LANE
HIGHFIELD LANE
ROSELANDS GDNS
HIGHFIELD HALL
24 hr RECEPTION Key Collection
NILE RD
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(University of Southampton)

Dear Colleague,

Re: The Journal of Bone and Joint Surgery

I am delighted to say that the British Volume of the Journal of Bone and Joint Surgery will continue to publish free the Abstracts of your Society's Meetings.

At the Journal of Bone and Joint Surgery, we believe it is very important to encourage all those involved in the practice of orthopaedic surgery to subscribe to the premier orthopaedic Journal.

In an age of specialisation, I appreciate the importance of speciality journals. Nonetheless, across the field of orthopaedics around the world many surgeons believe they should reserve their best work for submission to the JBJS. Within its pages, therefore, is the cream of world orthopaedic research.

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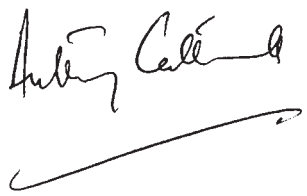
Yours sincerely,



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