4th Joint Meeting

of the

Bone Research Society

and the

British Orthopaedic Research Society

4-5 September 2013

Oxford, UK

Final Programme and Abstracts

Bone Research Society

www.brsoc.org.uk

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

BRS Annual Meeting 2014
Sheffield, 25-26 June 2014

BRS Clinical Training Course: Osteoporosis and Other Metabolic Bone Diseases
Oxford, 19-21 March 2014

For further details on all BRS activities and events please see **www.brsoc.org.uk**

British Orthopaedic Research Society

www.borsoc.org.uk

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

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

BORS Annual Meeting 2014
Bath, 23-24 June 2014

For further details on all BORS activities and events please see **www.borsoc.org.uk**

Contents

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BORS abstracts will be published in the *Bone and Joint Journal*; BRS abstracts will be published in *Frontiers in Bone Endocrinology*.

Supporters

The Bone Research Society and the British Orthopaedic Research Society are extremely grateful to the following companies who have agreed to support the meeting. With the exception of any company-sponsored satellite symposia, none of these companies has had any input into the content of the scientific programme.

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Scientific Programme

Tuesday 3rd Sept	BRS/BORS 2013 @ Botnar Seminars and concurrent workshops
14:00	New Investigators' Session **Working abroad: Should I stay or should I go, where to, and how do I come back?** with Adam Taylor and Celia Gregson and guest speaker Afsie Sabokbar, Director of Graduate Studies, NDORMS, University of Oxford
15:00	Seminar Chair: Jim Gallagher (Liverpool, UK) **Alan Boyde** (London, UK): Methods for assessing microstructure in bone organs
15:45	Concurrent Workshops Workshop 1 Chair: Andrew McCaskie (Newcastle-upon-Tyne, UK) **Andrew Carr** (Oxford, UK): Design and management of orthopaedic surgical trials Workshop 2 Chair: David Mahoney (Oxford, UK) **Jo Price** (Bristol, UK): Osteocytes – key mediators in bone homeostasis Workshop 3 Chair: Fraser Coxon (Aberdeen, UK) **Clarence Yapp** (Oxford, UK): Live confocal/multiphoton imaging in musculoskeletal tissues
16:30	Coffee
16:45	Concurrent Workshops Workshop 4 Chair: Kassim Javaid (Oxford, UK) **Gavin Clunie** (Cambridge, UK): Gaining advantage in the research and clinical management of rare metabolic bone disorders through the potential of disease registries Workshop 5 Chair: Jon Tobias (Bristol, UK) **Lynne Hocking** (Aberdeen, UK): Genetic basis of musculoskeletal disorders and assessment Workshop 6 Chair: Rob van 't Hof (Edinburgh, UK) **Kjell Laperre** (Bruker): uCT assessment of musculoskeletal tissues

Wednesday 4th Sept	At the Examination Schools, 75-81 High Street, Oxford OX1 4BG	
09:00-09:50	Registration and coffee Poster hanging	
09:50-10:00	*South School* Welcome and Opening Remarks **Andrew McCaskie (BORS President)/Tim Arnett (BRS President)**	
10:00-11:00	*South School* Symposium **Arthritis and musculoskeletal pain** Chairs: Andrew Carr (Oxford, UK)/Jim Gallagher (Liverpool, UK) IS1 **Irene Tracey** (Oxford, UK): Role of the CNS in controlling musculoskeletal pain IS2 **David Walsh** (Nottingham, UK): Assessment and management of musculoskeletal pain	
11:05-12:15	*South School* **Oral Communications** Chairs: Allie Gartland (Sheffield, UK)/Graham Russell (Oxford, UK) OC1 FRACTURE RISK PERCEPTION AND 3-YEAR INCIDENT FRACTURE RATES AMONG POSTMENOPAUSAL WOMEN WITH A RANGE OF CO-MORBID DISEASE: FINDINGS FROM THE GLOBAL LONGITUDINAL STUDY OF OSTEOPOROSIS IN WOMEN (GLOW) CL Gregson (Bristol, UK) OC2 PHOSPHO1: AN EMERGING ROLE IN ENERGY METABOLISM KJ Oldknow (Edinburgh, UK) OC3 DEFICIENCY OF MINERALIZATION INHIBITOR NPP1 PROTECTS AGAINST OBESITY AND DIABETES C Huesa (Edinburgh, UK)	*East School* **Oral Communications** Chairs: Richie Gill (Bath, UK)/Hamish Simpson (Edinburgh, UK) OC8 APPLICATION OF A NOVEL PERFUSION BIOREACTOR WITH INTEGRATED ULTRASOUND STANDING WAVE TRAP FOR AUGMENTATION OF CARTILAGE TISSUE ENGINEERING S Li (Southampton, UK) OC9 MORPHOLOGICAL CHARACTERISTICS OF CHONDROCYTES IN INJURED CARTILAGE CULTURED UNDER VARIOUS CONDITIONS A Karim (Edinburgh, UK) OC10 PROTECTION OF ARTICULAR CARTILAGE IN VIVO AGAINST SCALPEL-INDUCED INJURY BY A HYPEROSMOLAR SOLUTION NM Eltawil (Edinburgh, UK)

	OC4 REGULATION OF SKELETAL AND SOFT TISSUE MINERALISATION BY NPP1 (ECTO-NUCLEOTIDE PYROPHOSPHATASE PHOSPHODIESTERASE) MOR Hajjawi (London, UK) OC5 THE INHIBITORY ACTIONS OF ATP AND UTP ON BONE MINERALISATION ARE PARTIALLY MEDIATED BY THE ACTIVITY OF NPP1 (ECTO-NUCLEOTIDE PYROPHOSPHATASE/PHOSPHODIESTERASE 1) IR Orriss (London, UK) OC6 HIGH IMPACT EXERCISE SUBSTANTIALLY INCREASED THICKNESS OF THE SUPERIOR FEMORAL NECK CORTEX IN OLDER MEN, DESPITE ONLY MODEST CHANGES IN FEMORAL NECK AREAL BONE MINERAL DENSITY SJ Allison (Loughborough, UK) OC7 A GWAS IN AN EXTREME HIGH BONE MASS POPULATION SHOWS EXCESS SIGNAL FROM GENES ASSOCIATED WITH BMD IN THE NORMAL POPULATION CL Gregson (Bristol, UK)	OC11 STATIC AND DYNAMIC EFFECTS ON ARTICULAR CARTILAGE STUDIED WITH A NOVEL ISOLATED JOINT ORGAN CULTURE MODEL YC Lin (Edinburgh, UK) OC12 FACTORS AFFECTING THE EXTENT OF CHONDROCYTE DEATH DURING CARTILAGE DRILLING MMH Farhan-Alanie (Edinburgh, UK) OC13 ALTERATIONS IN SCLEROSTIN PROTEIN LEVELS IN CHONDROCYTES OF DIFFERENT PHENOTYPES FMD Henson (Cambridge, UK) OC14 ANALYSIS OF PROXIMAL FEMUR SYMMETRY USING STATISTICAL SHAPE MODELS BASED ON DATA FROM THE OSTEOARTHRITIS INITIATIVE C Lindner (Manchester, UK)
12:15-13:30	Lunch Annual General Meetings: BRS (South School) BORS (East School)	
13:30-14:30	*South School* Symposium **Cancer and bone** Chairs: Claire Edwards (Oxford, UK)/ Fraser Coxon (Aberdeen, UK) IS3 **Theresa Guise** (Indianapolis, USA): Clinical and translational elements of cancer bone disease	*East School* Symposium **Early osteoarthritis and joint preserving treatments** Chairs: Sion Glyn Jones (Oxford, UK)/Andrew McCaskie (Newcastle-upon-Tyne, UK) IS5 **Stefan Lohmander** (Lund, Sweden): Early osteoarthritis

	IS4 **Gabri van der Pluijm** (Leiden, Netherlands): Recent developments in the preclinical imaging of bone metastasis	IS6 **Richard Field** (Epsom, UK): Advances in joint preserving surgery of the hip
14:35-15:25	*South School* **Oral Posters** Chairs: James Edwards (Oxford, UK)/Debbie Mason (Cardiff, UK) OP1 POTASSIUM SUPPLEMENTATION AND BONE METABOLISM: A META-ANALYSIS H Lambert (Guildford, UK) OP2 THE EFFECTS OF METAL-ON-METAL HIP IMPLANTS ON BONE CELL BIOLOGY IN VIVO SSS Mahmoud (Cardiff, UK) OP3 TARGETING ADIPONECTIN INCREASES OSTEOBLAST AND CHONDROCYTE DIFFERENTIATION AND FUNCTION VIA BOTH ADIPONECTIN RECEPTOR-1 AND -2 J Carrick (Oxford, UK) OP4 LIPOSOMAL THERAPEUTIC DELIVERY SYSTEM FOR POLYMETHYL METHACRYLATE BONE CEMENT W Nishio Ayre (Cardiff, UK) OP5 STIMULATING ADIPONECTIN REDUCES BONE EXPRESSION OF NERVE GROWTH FACTOR IN MYELOMA-BEARING MICE; A NOVEL APPROACH TO COMBAT BONE PAIN IN CANCER-BONE DISEASE SWZ Olechnowicz (Oxford, UK) OP6 PATELLOFEMORAL JOINT MORPHOLOGY IN NORMAL AND REPLACED KNEES SJ Mellon (Oxford, UK) OP7 THE ROLE OF MICRORNAS IN FUNCTIONAL OSTEOMIMICRY IN PROSTATE CANCER CELLS SR Rao (Oxford, UK) OP8 BIOMECHANICAL CHANGES FOLLOWING HIGH TIBIAL OSTEOTOMY GM Whatling (Cardiff, UK) OP9 FUNCTIONAL SEGREGATION OF IMMUNOMODULATORY AND DIFFERENTIATION-COMPETENT MESENCHYMAL STROMAL CELL POPULATIONS SR James (York, UK)	

	OP10 TECHNICAL VALIDATION OF A NOVEL FLEXIBLE CAPACITIVE FORCE SENSOR FOIL M Mentink (Oxford, UK)	
15:25-16:25	Coffee and Attended Posters (odd numbers)	
16:25-17:25	*South School* Symposium **Vitamin D** Burden of vitamin D deficiency **Kassim Javaid** (Oxford, UK) Evidence for vitamin D therapy in early life: a systematic review **Nick Harvey** (Southampton, UK) NOS guidelines for management of vitamin D deficiency **Neil Gittoes** (Birmingham, UK)	*East School* **Oral Communications** Chairs: Bruce Caterson (Cardiff, UK)/Nikki Horwood (Oxford, UK) OC15 THE INNATE IMMUNE RESPONSE - A POTENTIAL THERAPEUTIC TARGET IN THE ACCELERATION OF FRACTURE REPAIR IN NORMAL AND OSTEOPOROTIC FRACTURES JK Chan (Oxford, UK) OC16 A ONE YEAR IN VIVO STUDY OF THE INFLAMMATORY RESPONSE TO A NOVEL BIORESORBABLE COMPOSITE PLATE FOR THE FIXATION OF BONE FRACTURES AL Mohamad (Nottingham, UK) OC17 ROLE OF PERIVASCULAR STEM CELLS IN THE PREVENTION OF ATROPHIC NONUNION T Tawonsawatruk (Edinburgh, UK) OC18 COULD DIFFERENT SHAPES OF NANOSIZED HYDROXYAPATITE HAVE A ROLE IN MODULATING BONE FORMATION? P Kalia (London, UK) OC19 THE INFLUENCE OF COMPOSITE SCAFFOLD POROSITY ON CELL DIFFERENTIATION AND ECM PRODUCTION: BONE VS. CARTILAGE P Kalia (London, UK) OC20 INVESTIGATING THE EFFECTS OF DEMINERALISED BONE MATRIX ON TENDON-BONE HEALING AND AUGMENTATION OF TENDON LENGTH IN AN IN VIVO MODEL. CH Holden (London, UK)

17:30-18:00	*South School* **Charles Dent Lecture** Chair: Tim Arnett (London, UK) **Graham Russell** (Oxford/Sheffield, UK)
18:00-19:00	*South School* **Satellite Symposium** Sponsored by Servier Chair: Juliet Compston (Cambridge, UK) Kassim Javaid (Oxford, UK): Strontium – How does it really work? Ed Middleton (Hull, UK): Treatment after bisphosphonates – Where is the evidence? Mike Stone (Cardiff, UK): What else can Strontium offer osteoporotic patients?
19:00	End
19:15 for 19:45	Conference Dinner Wadham College Parks Road, Oxford OX1 3PN

Thursday 5th Sept	At the Examination Schools, 75-81 High Street, Oxford OX1 4BG	
08:30-10:00	*South School* **Oral Communications** (Basic) Chairs: Theresa Guise (Indianapolis, USA)/Isabel Orriss (London, UK) OC30 NOVEL MICROCT-BASED METHOD TO ASSESS THE 3D STRUCTURE OF CARTILAGE, SUBCHONDRAL BONE AND OSTEOPHYTES SIMULTANEOUSLY IN MURINE OSTEOARTHRITIS PN Borges (London, UK) OC31 NBQX, AN AMPA/KAINATE GLUTAMATE RECEPTOR ANTAGONIST, ALLEVIATES JOINT PATHOLOGY AND BONE REMODELLING IN ARTHRITIS CS Bonnet (Cardiff, UK) OC32 DEVELOPMENT OF NOVEL MODEL SYSTEMS TO REVEAL THE ROLE OF PHOSPHO1 IN THE INITIATION OF SKELETAL MINERALISATION DA Houston (Edinburgh, UK) OC33 AUGMENTATION OF OSTEOCLASTOGENESIS IN THE ABSENCE OF THE P2X7 RECEPTOR IN OESTROGEN DEPLETE CONDITIONS IN VITRO IS DEPENDENT UPON PRECURSOR CELL ORIGIN. A Gartland (Sheffield, UK) OC34 P2X7 RECEPTOR POLYMORPHISMS MODULATE OSTEOBLAST CELL FUNCTIONS A Gartland (Sheffield, UK) OC35 LOSS OF P58IPK, THE CELLULAR INHIBITOR OF PKR AND PERK, CAUSES BONE CHANGES AND JOINT DEGENERATION IN MICE. DJ Mason (Cardiff, UK)	*East School* **Oral Communications** (Clinical) Chairs: Gavin Clunie (Cambridge, UK)/Iain McNamara (Nottingham, UK) OC21 EFFECT OF METAL IONS AND PARTICLES ON OSTEOBLAST MINERALISATION ON GRIT BLASTED, TITANIUM COATED AND HYDROXYAPATITE COATED PROSTHESIS SURFACES KM Shah (Sheffield, UK) OC22 STABILISING CARTILAGE TO PROTECT AGAINST OSTEOARTHRITIS PATHOLOGY: LESSONS FROM LONG BONE DEVELOPMENT KA Staines (London, UK) OC23 PREVENTING INFECTION AROUND OSSEOINTEGRATED TRANSCUTANEOUS AMPUTATION PROSTHESES USING ELECTROCHEMICALLY DEPOSITED HYDROXYAPATITE AND SILVER M Chimutengwende-Gordon (London, UK) OC24 IN VIVO ASSESSMENT OF POROUS IMPLANTS FUNCTIONALISED WITH RGD PEPTIDES IN ENHANCING OSSEOINTEGRATED AMPUTATION PROSTHESIS BJ Thomas (London, UK) OC25 OESTRADIOL AND DHEA MAY INFLUENCE THE DEVELOPMENT OF OSTEONECROSIS IN ADOLESCENTS BEING TREATED WITH CORTICOSTEROIDS MR Adams (Cardiff, UK) OC26 A HISTOMORPHOMETRIC AND BIOMECHANICAL EVALUATION OF THE OSSEOINTEGRATION OF LASER-ETCHED IMPLANTS IN AN

	OC36 OBESITY PROMOTES THE DEVELOPMENT OF MULTIPLE MYELOMA AND THE ASSOCIATED BONE DISEASE IN VIVO ST Lwin (Oxford, UK) OC37 EVIDENCE OF A MYELOMA CELL NICHE: DORMANCY IS AN ACQUIRED STATE MA Lawson (Sheffield, UK) OC38 ZOLEDRONIC ACID AFFECTS OSTEOBLASTS IN VIVO WITH POTENTIAL IMPLICATIONS FOR THE BONE METASTASIS NICHE HK Brown (Sheffield, UK)	OVINE MODEL. K Erskine (London, UK) OC27 PREVALENCE OF RADIOGRAPHIC HIP OSTEOARTHRITIS IS INCREASED IN HIGH BONE MASS: A CASE-CONTROL STUDY SA Hardcastle (Bristol, UK) OC28 WEAR OF A ROTATING HINGE KNEE JOINT J Meswania (London, UK) OC29 CHILDHOOD BONE SIZE, MINERALISATION AND DENSITY ARE ASSOCIATED WITH METHYLATION STATUS OF THE CDKN2A PROMOTER AT BIRTH NC Harvey (Southampton, UK)
10.00-11.00	Coffee and Attended Posters (even numbers)	
11:00-12:00	*South School* Symposium **Fracture** Chairs: Ken Poole (Cambridge, UK)/Mark Thompson (Oxford, UK) IS7 **Georg Duda** (Berlin, Germany): Convergence of biology and mechanics in musculoskeletal regeneration IS8 **Peter Smitham** (London, UK): Fracture healing- the knowns and some known unknowns	*East School* **Oral Communications** Chairs: Mark Birch (Newcastle-upon-Tyne, UK)/Eugene McCloskey (Sheffield, UK) OC39 THE NITRIC OXIDE SYNTHASE INHIBITOR L-NAME BLOCKS THE INCREASE IN LIMB PERFUSION INDUCED BY PARATHYROID HORMONE AND REDUCES THE ANABOLIC EFFECT OF PTH ON BONE S Gohin (London, UK) OC40 SHOULD BISPHOSPHONATE THERAPY BE SUSPENDED AT THE TIME OF FRACTURE IN PATIENTS TREATED WITH A DIRECT BONE HEALING STRATEGY? T Savaridas (Edinburgh, UK) OC41 MATERNAL 25(OH)-VITAMN D STATUS IN LATE PREGNANCY IS ASSOCIATED WITH OFFSPRING MUSCLE STRENGTH IN EARLY CHILDHOOD RJ Moon (Southampton, UK)

		OC42 ENHANCED MINERALISED MATRIX DEPOSITION ON LASER-ETCHED/HYDROTHERMALLY TREATED TITANIUM SURFACES RJM Morrison (Newcastle-upon-Tyne, UK) OC43 VITAMIN D STATUS IN OBESITY: EVALUATION OF FREE 25(OH)D AL Evans (Sheffield, UK) OC44 COMBINING STATISTICS-BASED RAMAN SPECTROSCOPY AND QUANTITATIVE TWO-PHOTON MICROSCOPY FOR ROUTINE NON-INVASIVE OSTEOCHONDRAL IMAGING P Kalia (London, UK)
12:05-12.40	*South School* **Oral Posters** Chairs: Brigitte Scammell (Nottingham, UK)/Kate Ward (Cambridge, UK) OP11 DOES PHYSICAL ACTIVITY INFLUENCE ADOLESCENTS BONE MINERAL DENSITY WITH EQUAL EFFECT AT ALL BMI LEVELS? FIT FUTURES 2010-2011, THE TROMSØ STUDY A Winther (Tromsø, Norway) OP12 ASSESSING SURGICAL OUTCOMES WITH INDUSTRY METRICS: A PROSPECTIVE COHORT STUDY OF 6912 ARTHROPLASTY PATIENTS DF Hamilton (Edinburgh, UK) OP13 REDUCED EXERCISE BENEFITS IN BONE IN OLDER AGE AND IN EXERCISE BEGUN IN ADULTHOOD AD Ireland (Manchester, UK) OP14 MEASURING BONE MINERAL DENSITY OF THE PROXIMAL FEMUR FOLLOWING TOTAL HIP ARTHROPLASTY USING NOVEL, REGION-FREE DUAL ENERGY X-RAY ABSORPTIOMETRY ANALYSIS SOFTWARE (DXA-RFA) RM Morris (Sheffield, UK) OP15 CHARACTERISING MUSCULOSKELETAL PHENOTYPE IN A POPULATION WITH LOW FRACTURE INCIDENCE KA Ward (Cambridge, UK) OP16 CESSATION OF AMBULATION, NOT STEROID EXPOSURE RESULTS IN A DRAMATIC LOSS OF TRABECULAR BONE DENSITY IN BOYS WITH	

	DUCHENNE MUSCULAR DYSTROPHY (DMD) NJ Crabtree (Birmingham, UK) OP17 3D MENISCUS KINEMATICS THROUGHOUT KNEE FLEXION AND LOADING: A NOVEL IN VIVO MRI STUDY OF THE KNEE DJ Watling (Cardiff, UK)	
12:40-13:30	Lunch and Posters	
13:30-14:15	*South School* **Keynote Lecture** Chair: Cyrus Cooper (Southampton/Oxford, UK) IS9 **Steve Cummings** (San Francisco, USA): The biology of aging - how it applies to bone and muscle	
14:15-15:15	*South School* **Debate: 'Bisphosphonate therapy should be discontinued after 5 years'** Supported with an unrestricted educational grant by Merck Chairs: Celia Gregson (Bristol, UK)/Roger Smith (Oxford, UK) **FOR THE MOTION: Juliet Compston** (Cambridge, UK) **AGAINST THE MOTION: Eugene McCloskey** (Sheffield, UK)	
15:15-15:35	Coffee	
15:35-16:35	*South School* Symposium **Clinical Update** Chairs: Steven Cummings (San Francisco, USA)/Kassim Javaid (Oxford, UK) IS10 **Peter Taylor** (Oxford, UK): Rheumatoid arthritis and associated bone disease IS11 **Cyrus Cooper** (Southampton/Oxford, UK): Osteoporosis	*East School* **Oral Communications** (Basic) Chairs: Adam Taylor (Lancaster, UK)/Jim Triffitt (Oxford, UK) OC45 FUNCTIONALIZED NOVEL SURFACES TO INFLUENCE MESENCHYMAL STEM CELLS SB Walsh (Newcastle-upon-Tyne, UK) OC46 BMP-9 INDUCES THE CALCIFICATION OF VASCULAR SMOOTH MUSCLE CELLS D Zhu (Edinburgh, UK) OC47 ENDOTHELIAL CELLS ACCELERATE THE OSTEOGENIC DIFFERENTIATION OF PERICYTES IR Murray (Edinburgh, UK)

		OC48 ATP AND UTP ARE POTENT INHIBITORS OF VASCULAR CALCIFICATION IR Orriss (London, UK) OC49 CAN SELECTED AND EXPANDED BONE MARROW STROMAL CELLS MAKE NEW BONE :A RANDOMISED CONTROL STUDY A Bhattacharjee (Oswestry, UK) OC50 ENHANCED OSTEOGENIC INDUCTION OF EMBRYONIC BONE DEVELOPMENT BY VASCULAR ENDOTHELIAL GROWTH FACTOR JM Kanczler (Southampton, UK)
16:40-16:50	*South School* **Awards**	
16:50	End of meeting	

IS1

Role of the CNS in controlling musculoskeletal pain

Irene Tracey Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) & Nuffield Division Anaesthetics, Nuffield Department Clinical Neurosciences, University of Oxford, UK

The past decade has seen a rapid increase in the number of papers published in the area of 'pain imaging'. Many techniques are now available to explore the human central nervous system from a functional, structural and chemical perspective in both patients and healthy subjects. Relating specific neurophysiologic measures to perceptual or non-perceptual changes induced by peripheral or central sensitisation, behavioural, psychological or pharmacological mechanisms and identifying their site of action within the CNS has both value and has been a major goal for scientists, clinicians and the pharmaceutical industry. Identifying non-invasively where functional and structural plasticity, sensitisation and other amplification or attenuation processes occur along the pain neuraxis for an individual and relating these neural mechanisms to specific pain experiences, measures of pain relief, persistence of pain states, degree of injury and the subject's underlying genetics, has neuroscientific relevance and potential diagnostic value. With the advent of functional neuroimaging methods, such as Blood Oxygen Level Dependent (BOLD) functional magnetic resonance imaging (FMRI), Arterial Spin Labelling (ASL) quantitative perfusion imaging, positron emission tomography (PET), electroencephalography and magnetoencephalography this has been made feasible. These read-outs of varying physiological types are not simple surrogate measures of pain rating, but rather a complex and sophisticated non-invasive 'behind the scenes' neurophysiological read-out of nociceptive processing that underpins the subjective experience and can be powerfully used to aid explanation of a subject's multidimensional pain experience. As the measures can be related to what the subject describes and what we can additionally measure about the subject (psychological, personality, physiological), it enables us to disentangle for an individual how or whether factors like anxiety, depression, attention, central sensitization, etc., mechanistically might influence nociceptive processing at the brain level to alter the pain experience. It is my expectation that 'pain neuroimaging' will play an increasing role in pain neuroscience, clinical decision-making and analgesic drug development in the coming decade.

IS2

Assessment and management of musculoskeletal pain

Professor David A. Walsh Director Arthritis Research UK Pain Centre, Honorary Consultant Rheumatologist Academic Rheumatology, University of Nottingham Clinical Sciences Building, City Hospital, Hucknall Road, Nottingham, NG5 1PB

Chronic musculoskeletal pain is a major cause of disability and distress in our ageing population. Far from being a single diagnosis, pain results from complex interactions between musculoskeletal pathology, peripheral and central nociceptive processing, and the psychosocial context in which pain is experienced. Pain assessment brings together the traditional clinical skills of history and examination, with special investigations addressing both the origins and mechanisms of pain. Pain is not a single entity, and the individual with arthritis will typically describe multiple pains that may be constant or intermittent, burning or throbbing, mild or severe, bothersome or accepted. Management requires exploration of how pain impacts on valued activities, elucidating pain mechanisms, and identifying barriers to recovery and predictors of pain progression. Existing treatments may be effective at relieving chronic musculoskeletal pain, but pain relief is often incomplete, and comes at the cost of significant adverse events. Recent advances in the management of musculoskeletal pain have resulted from a better understanding of the relationship between structural pathology and symptoms, the power of the central nervous system to moderate pain signals, and the development of evidence-based stratification tools that facilitate optimal outcomes in a health economic environment with limited resources.

IS3

Clinical and translational elements of cancer bone disease

Theresa A. Guise, M.D.

Bone metastases cause significant morbidity and once housed in bone, the tumors are incurable. Tumors produce factors which stimulate osteoclasts and osteoblasts to dysregulate normal bone remodeling. The bone microenvironment alters the behavior of metastatic tumor cells, driving a feed-forward cycle that makes skeletal metastases refractory to treatment and cure. Transforming growth factor beta (TGFβ) is a central factor in this vicious cycle. It is deposited into mineralized bone matrix by osteoblasts, released and activated by osteoclastic bone resorption, and changes the phenotype of tumor cells.

In mouse models, TGFβ blockade inhibits osteolytic bone metastases due to breast cancer prostate cancer and melanomas by blocking tumor-produced osteolytic and prometastatic factors (PTHrP, IL-11, CTGF). It also increases bone mass, independent of effects on cancer cells, by increasing osteoblast activity and reducing osteoclast activity. These effects are potentiated with the use of a bisphosphonate, zoledronic acid.

Since the bone microenvironment is hypoxic, we tested the interaction between TGFβ and hypoxia signaling. We found that bone metastases are hypoxic and 1% O2 increases hypoxia-inducible factor (HIF)1α in MDA-MB-231 breast cancer cells. Combined treatment with 1% O2 and TGFβ additively increased mRNA expression and promoter activity of prometastatic factors VEGF and CXCR4, suggesting that HIF1α promotes bone metastasis via crosstalk with TGFβ. Our results show that hypoxia/HIF1α signaling promotes bone metastasis, which were inhibited when preventively blocking HIF1α through genetic or pharmacologic approaches. Bone metastases development was further inhibited when targeting both HIF1α and TGFβ signaling.

In contrast to beneficial findings of TGFβ blockade in osteolytic bone metastases, TGFβ signaling blockade increases growth of the osteoblastic prostate xenograft LuCAP23.1. These effects are likely due to the direct effect of TGFβ blockade to alter the host response by increasing osteoblast differentiation. Taken together, these data suggest that inhibition of TGFβ signaling may be effective in osteolytic disease, but may accelerate osteoblastic bone metastases due to its effects to stimulate osteoblast activity.

Other tumor mediators of osteoblastic metastases have been implicated, such as endothelin-1 (ET-1), and are targets for therapy. Tumor produced-ET-1 stimulates osteoblast activity via the endothelin A receptor (ETAR). ETAR blockade abrogates osteoblastic disease in mouse models and improves survival in men with prostate cancer metastases to bone. ET-1 suppresses osteoblast production of the Wnt pathway inhibitor DKK1 to result in the dysregulated new bone formation associated with osteoblastic disease. Furthermore, blockade of ETAR is associated with reduced bone mass in bone unaffected by tumor.

In summary, tumor bone microenvironment is rich in factors that cause cancer cells to thrive. Blockade of these factors have important implications for the skeletal health of cancer patients. The result can improve bone metastases, but may have differential effects which depend on the osteolytic or osteoblastic metastatic phenotype. Such therapy may alter bone remodeling at sites unaffected by tumor.

IS4

Recent developments in the preclinical imaging of bone metastasis

Gabri van der Pluijm, PhD. Leiden University Medical Centre, Dept. of Urology J3-100, Albinusdreef 2, 2333ZA Leiden, The Netherlands

Imaging has become an indispensable tool in cancer biology and in clinical practice. Recent advances in molecular biology and imaging technology have facilitated the successful introduction of different imaging modalities for small animals as preclinical models of human disease. During the past decade an increasing number of biomedical imaging techniques have been implemented in preclinical bone metastasis models for sensitive real-time cancer cell tracking, bone homing (functional studies) and the monitoring of drug response (experimental therapeutics). These imaging techniques include optical imaging (fluorescence and bioluminescence imaging; FLI, BLI, multi-photon microscopy), microCT, PET, MRI and SPECT. During my presentation recent developments in the real-time in vivo imaging of cancer metastasis to bone will be discussed. It is important to note that no ideal imaging modality exists that combines excellent spatial resolution, cancer cell detection sensitivity and quantification. The choice of equipment and imaging strategy, therefore, depends on the objectives of the study. For instance, novel, improved reporter genes/constructs (e.g. codon-optimized luciferases) allow extremely

sensitive cancer cell tracking (< 50 cancer cells in bone is feasible). Furthermore, it is possible to visualize -in real-time- the expression and activity of specific molecules (e.g. receptors, proteases by activatable imaging probes), growth factor pathway activities and biological processes (e.g. angiogenesis, apoptosis, bone turnover). Last-but-not-least, molecular imaging has strongly facilitated the development of new therapy for the treatment of skeletal metastasis. Molecular imaging of cancer growth and bone metastasis in preclinical models thus provides the essential link between cell-based experiments and clinical translation.

IS5

Early osteoarthritis

L Stefan Lohmander Senior Professor, Department of Orthopaedics, Clinical Sciences Lund, Lund University, Sweden and Professor, Research Unit for Musculoskeletal Function and Physiotherapy and Department of Orthopaedics and Traumatology, University of Southern Denmark

Osteoarthritis is characterized structurally by focal areas of damage to the articular cartilage on load-bearing joint surfaces, associated with new bone formation at the joint margins, changes in the subchondral bone, and variable degrees of synovitis. It is caused by a mixture of systemic factors that predispose to the disease, and local mechanical factors that dictate its distribution and severity. When the disease is advanced it is visible on plain radiographs, which show narrowing of joint space due to cartilage loss, osteophytes, and changes in the subchondral bone. These changes in joint structure reflect advanced stages of joint pathology associated with osteoarthritis development. Commonly, the disease has been active for many years when these changes are visible on plain X-ray examination. With the use of MRI we are now able to visualize earlier stages of osteoarthritic joint pathology in cartilage, synovium, bone and other joint structures. The patient-reported problems associated with these pathological changes include joint pain related to use, short-lasting inactivity stiffness of joints, pain on movement with a restricted range, and crepitus. Pain is the particularly important symptom leading to health-care consultation. However, the correlation between structural evidence of osteoarthritis detected by radiographs or MRI and the symptomatic disease is weak. This raises issues relating to the definition of the 'disease' and the 'illness' of osteoarthritis, and the focus of management and prevention of disease and disease progression. Perhaps structural joint changes should in this context be regarded as risk factors for the illness. The natural history of either the 'disease' (the pathological tissue changes) or the 'illness' (the symptoms) of osteoarthritis remains incompletely understood. A better understanding of, and ability to predict, the trajectory of the early stages of osteoarthritic disease as well as illness is required before we introduce for general use interventions aimed to modify osteoarthritis risk and development

IS6

Advances in joint preserving surgery of the hip

Richard Field, Epsom

Joint preserving surgery of the hip is generally undertaken to alleviate of symptoms caused by joint damage. While there is a growing body of literature linking both genetic and anatomic variations with the development of hip pathology, screening techniques that reliably predict which individuals will become symptomatic or at what stage their hips will progress to joint failure remain to be validated in clinical practice. In consequence, prophylactic interventions, prior to the onset of symptoms or pathological change remain untested. Furthermore, the results of joint preserving surgery of the hip remain operator dependent and the boundary between reversible and irreversible damage remains undefined. In this environment of uncertainty, a spectrum of new pathologies and treatments are being reported.

Advances in our understanding of joint pathology include the development of the suction seal concept, the development of models for dynamic joint lubrication and joint damage when the suction seal mechanism is compromised.

In femoroacetabular impingement surgery, robust evidence is emerging to demonstrate that arthroscopic and mini-open techniques achieve equal or greater clinical benefit to open joint disarticulation, with less co-morbidity. Clinical studies are being undertaken to determine whether femoro-acetabular impingement surgery is more effective than activity modification and physical therapy, whether labral repair or reconstruction is better than labral resection, whether fibrin glue re-fixation can preserve hyaline articular cartilage and whether debridement, microfracture or grafting is the best treatment for areas of acetabular articular cartilage loss.

In the management of hip dysplasia, techniques to undertake peri-acetabular osteotomy have been refined to perform surgery more quickly, with less blood loss, with less soft tissue trauma and without inflicting unsightly scars.

A growing number of surgeons are using motion analysis studies to identify the bone that need to be removed to relieve femoro-acetabular impingement, to aid their planning of realignment osteotomies, to identify sub-spinous impingement and to ensure that adequate femoral head coverage and joint stability are preserved.

The use of stem cells, platelet rich plasma and growth hormones are being investigated to determine whether the symptoms of degenerative disease can be relieved and whether hyaline articular cartilage regeneration may be achieved.

IS7

Convergence of biology and mechanics in musculoskeletal regeneration

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The initial events after fracture seem to be essential for fast and uneventful bone healing. Tissue organization (e.g. invasion of vessels) starts early, leading to a restructuring of the hematoma. Interestingly, almost all accounts of the fracture healing process begin with the discussion of the invasion of inflammatory cells to the wound site as the first event. However, this starting point ignores the important fact that the initial fracture hematoma already contains inflammatory cells. The initial cell composition and its particular microenvironment could be among the factors, which crucially determine whether fracture healing proceeds effectively. This talk aims to give a detailed overview, beginning with the first events in fracture healing and bone regeneration, followed by a description of the close interplay between bone and the immune system, which already implicates an important role for immune cells in fracture healing. Bone healing in knockout mice devoid of mature T and B cells is compared with the healing in mice depleted of cytotoxic (CD8) T cells at the time of injury. The data is considered in the context of in vitro results and compared to a large animal model as well as clinical studies. Results focus on aspects of the early fracture hematoma with special regard to the initial immunologic features. We will demonstrate the importance of immune cells for the cellular composition in the injured region dictating the healing course and progression. This research seems to be of vital importance, especially in immunologically suppressed patients, suffering from delayed healing or non-union situations. The understanding of the initial phase of healing could play a pivotal role in future fracture treatment strategies and allow us to differentiate between aspects of inflammation that are essential for bone healing and those that delay bone healing.

IS8

Fracture healing - the knowns and some known unknowns

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Understanding fracture healing provides the rather unique situation of studying an organ that can heal itself. It could provide information that may potentially be extrapolated to other tissues and has generated particular interest in certain signalling pathways and pluripotent stem cells. There are however several distinct therapeutic challenges to consider. These include: investigating methods to accelerate fracture healing; prevention or treatment of non-union; treatment of large

segmental defects; spinal fusion and other arthrodesis; osteointegration of implants both primary and in revision arthroplasty or other situations of lost bone stock and understanding the impact genetics and epigenetics may have in terms of personalised medicine. All these are on the backdrop of a global problem of road traffic accidents causing high energy fractures, a pandemic of osteoporosis leading to fragility fractures and an expected 400% rise in arthroplasties within the next 10 years. Healing bone is therefore a problem that is only going to get bigger. We know that fracture healing is influenced by a number of factors including angiogenesis, growth factors, inflammation, structural scaffold, cell maintenance, tissue formation and the nervous system. The mechanical environment which they encompass affects all of these and therefore must be considered in conjunction with all the above and yet the optimal mechanical condition changes through the stages of fracture healing. Any new treatment options must understand these factors and possibly stratify treatment options according to certain goal requirements. We know that for any treatment to reach human trials it must demonstrate efficacy in animal models. This has led to numerous animal models being developed each having different advantages and disadvantages. However, the challenge has been demonstrating such advantages in clinical trials where the complexities of human lifestyles and the difficulty of determining hard endpoints can muddy the water. More than ever before, treatment options must also demonstrate cost-effectiveness in patients. Meta-analysis of trials is challenging because of the heterogeneity of various trials and device specifications in both animal and human studies. The future may involve investigating combinations of therapies and developing a consensus on future trial methods.

IS9

The biology of aging - how it applies to bone and muscle

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Bone and muscle age and lose function as part of a larger process of aging of the whole body. Aging results from the accumulation of disorder and damage with time. This comes from three main sources: - Entropy: the development of disorder with time. This law of thermodynamics applies to all things, notably muscle tissue. - Fatigue damage: microscopic damage develops in all materials, like bone and muscle, that undergo repetitive loading. - Chemical damage: molecular reactions occur with irreversible loss of function and strength. Cells require oxidative phosphorylation in mitochondria to produce ATP energy and that generates 'reactive oxygen species' (ROS) that do much of the damage to components of cells, especially mitochondria. Aging is slowed repairing damage or regenerating cells or tissues. There are two broad types: - Stem cells: an extracellular response to damage, they reproduce and differentiate to replace of short-lived cells (osteoclasts and osteoblasts) and tissues, like muscle. The ability of stem cells to replace tissue decreases with aging. - Autophagy: an intracellular process clears and recycles damaged proteins and organelles. This is particularly important for long-lived 'post-mitotic' cells such as osteocytes. Autophagy decreases with aging. Aging also results from progressive decrease in the ability to generate ATP energy from mitochondria. This process leads to slowing and weakness with aging in humans. Recent studies show that blocking autophagy in osteocytes accelerates age-related and preserving mitochondria content of muscle with aging prevents loss of muscle and bone and prolongs life. These pathways are novel and productive directions for fundamentally slowing the loss of bone and muscle strength with aging.

IS10

Rheumatoid arthritis and associated bone disease

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Rheumatoid arthritis is best thought of as a systemic inflammatory syndrome with its major manifestation in joints. It is heterogeneous in its presentation and evolution. Synovitis is a characteristic feature of this syndrome. The term pannus refers to synovitis that is adherent to cartilage and locally invasive where this arises, bone destruction occurs. Recognition of the destructive potential of synovitis is important because this process can be halted completely whereas once bone damage has occurred, it cannot (at the present time) be reversed. Where structural damage to joints occurs, it is cumulative over time and contributes significantly to functional decline. However, it has emerged in recent years that certain subgroups of rheumatoid arthritis subjects meeting contemporary classification criteria do not exhibit progressive structural damage. Furthermore, there is evidence that at a cohort level, the rate of radiographic damage has been declining in rheumatoid populations over recent years. There is speculation that this cannot be attributed solely to more effective use of synthetic convention disease modifying drugs or to the increasing use of biologics. Changes in smoking may also be another factor. Prognostic markers of bone loss have been identified and in some cases, the underlying biology responsible for this destructive link is well understood. One example is the link between the majority subtype of the syndrome in whom antibodies to cyclic citrullinated peptides may be detected and for which recent evidence suggests that the antibodies directly promote bone loss. The importance of signalling through the RANK ligand pathway is also well established.

IS11

Clinical update: Osteoporosis

Cyrus Cooper, MRC Lifecourse Epidemiology Unit, University of Southampton; and Institute of Musculoskeletal Sciences, University of Oxford, UK

Osteoporosis constitutes a major public health problem through its association with age related fractures. These fractures typically occur at the hip, spine and distal forearm. It has been estimated from incidence rates derived in North America that the lifetime risk of a hip fracture in Caucasian women is 17.5%, with a comparable risk in men of 6%. Age and sex-adjusted hip fracture rates are generally higher in Caucasian than in Asian populations. Furthermore, the pronounced female preponderance in fracture incidence observed in white populations is not seen amongst blacks or Asians in whom age-adjusted female to male incidence ratios approximate unity. Life expectancy is increasing around the globe and the number of elderly individuals is rising in every geographic region. Assuming constant age-specific incidence rates for fracture, the number of hip fractures occurring worldwide among people aged 65 years and over will rise from 1.66 million in 1990 to 6.26 million in 2050. Studies performed in the United States, Scandinavia, and the United Kingdom, between 1930 and the late 1980s, consistently reported increases in the age-adjusted incidence of hip fractures among men and women. This increase appears to have levelled off, in the northern regions of the United States, as well as more recently in Europe. Rates in Asian populations continue to show substantial rises between the 1960s and the present time. Algorithms for cost-effective risk assessment and treatment with a variety of pharmacological interventions are now available, and will serve to consolidate the downward secular tendency.

OC1

FRACTURE RISK PERCEPTION AND 3-YEAR INCIDENT FRACTURE RATES AMONG POSTMENOPAUSAL WOMEN WITH A RANGE OF CO-MORBID DISEASE: FINDINGS FROM THE GLOBAL LONGITUDINAL STUDY OF OSTEOPOROSIS IN WOMEN (GLOW)

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Patients with improved health understanding have greater autonomy over, and motivation towards, health-related lifestyles and treatment concordance. We compared self-perceived fracture risk and 3-year incident fracture rates in postmenopausal women reporting a range of co-morbid diseases using data from the Global Longitudinal study of Osteoporosis in Women (GLOW).

The GLOW study is an international cohort study involving 723 physician practices across 10 countries in Europe, North America, and Australia. At baseline 60,393 women aged >55 years completed questionnaires detailing medical history, including co-morbidities, fractures and self-perceived fracture risk. Annual follow-up then determined self-reported incident fractures.

In total, 2945/43832 (6.7%) sustained an incident fracture over 3 years. Co-morbidities were common: 86% reported >1 co-morbidity, most commonly hypercholesterolemia (50%) and hypertension (49%). All co-morbidities were strongly associated with increased fracture rates, particularly Parkinson's disease (hazard ratio [HR] 95% CI; 3.89 [2.78, 5.44]), multiple sclerosis 2.70 [1.90, 3.83], cerebrovascular events 2.02 [1.67, 2.46], and rheumatoid arthritis 2.15 [1.53, 3.04], all $p<0.001$). Accumulation of co-morbidities conveyed an incrementally increasing fracture rate with >/=4 comorbidities associated with a doubling of fracture risk. Most individuals perceived their own fracture risk to be similar to (46%) or lower than (36%) women of the same age.

Increased self-perceived fracture risk was strongly associated with incident fracture rates. However, only 29% of women who experienced a fracture had perceived their risk as increased. Under-appreciation of fracture risk occurred for all co-morbidities, including women with neurological diseases, in whom women with self-perceived low fracture risk had a fracture HR of 2.39 [1.74, 3.29] compared with women without co-morbidities.

Our results suggest postmenopausal women with co-morbidities known to be associated with increased fracture rates tend to under-appreciate their risk, including in the context of neurological diseases, where fracture rates are highest. Our findings support: updating of guidelines, particularly in relation to neurological diseases (Parkinson's disease, multiple sclerosis, and stroke); prompting fracture risk assessment by non-bone specialists, including primary care physicians; patient education in relation to bone health; and raising awareness among patient societies.

OC2

PHOSPHO1: AN EMERGING ROLE IN ENERGY METABOLISM

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Advances in genetic approaches to bone physiology have expanded our understanding of the mechanisms by which bone and energy homeostasis interact. PHOSPHO1, a bone specific phosphatase is essential for the initiation of bone mineralization. Here we now show that Phospho1 ablation confers a remarkable protection against obesity and diabetes in mice. To understand the mechanism whereby Phospho1 impacts metabolism, microarray analysis of osteoblasts, the primary site of Phospho1 expression was performed. Esp (encoding the phosphatase OST-PTP) which controls hormonally active osteocalcin secretion, was 20-fold more highly expressed in Phospho1-/- osteoblasts ($p<0.05$). Conversely, Esp mRNA was decreased in Phospho1 overexpressing osteoblasts ($p<0.001$). Unexpectedly, serum levels of uncarboxylated and undercarboxylated osteocalcin were normal suggesting an osteocalcin-independent mechanism of PHOSPHO1 regulated energy metabolism. 120 day-old Phospho1-/- mice were hypoglycaemic (wild-type (WT) 9.48±0.31 mmol/L, Phospho1-/- 8.30±0.26 mmol/L; $p<0.01$) and showed improved glucose and insulin tolerance compared to WT mice ($p<0.05$). These observations were consistent with the finding of smaller (mg/g BW) subcutaneous (WT 4.51±0.37, Phospho1-/- 2.79±0.42; $p<0.01$), mesenteric (WT 13.2±1.34, Phospho1-/- 5.56±1.61; $p<0.01$) and epididymal (WT 13.7±1.81, Phospho1-/- 6.96±0.58; $p<0.001$) fat deposits noted in Phospho1-/- mice at necropsy and confirmed by MRI and CT. Remarkably, Phospho1-/- mice resisted the pronounced weight gain (WT 38.0±1.54 g, Phospho1-/- 32.4±1.26 g; $p<0.05$) and diabetes (WT 10.3±0.53 mmol/L, Phospho1-/- 9.27±0.77 mmol/L; $p<0.05$) exhibited by WT mice when fed a chronic high fat diet (HFD; 14 weeks, 58% kcal as fat) and this was not explained by altered activity. Circulating levels of total and high molecular weight adiponectin were decreased in Phospho1-/- mice on both control and HFD, whereas leptin levels were decreased on HFD only ($p<0.05$). Histology revealed smaller epididymal adipocytes, decreased fat content, decreased pancreatic islet number and increased mitochondria number in brown fat ($p<0.05$). However, no differences were observed in brown fat specific genes including Ucp1 suggesting canonical thermogenesis does not underlie metabolic protection. Our findings indicate Phospho1 deficiency improves the metabolic profile of mice in vivo and confers resistance to obesity and diabetes most likely through a primary effect on bone metabolism/turnover.

OC3

DEFICIENCY OF MINERALIZATION INHIBITOR NPP1 PROTECTS AGAINST OBESITY AND DIABETES

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Bone has recently emerged as a novel endocrine organ regulating glucose metabolism. Ectonucleotide pyrophosphatase/phosphodiesterase-1 (NPP1) controls bone mineralisation by generating the mineralisation inhibitor pyrophosphate. In clinical studies increased activity of NPP1 has been found in patients with insulin resistance, and it has been shown to directly inhibit the insulin receptor. We hypothesised that mice lacking NPP1 (Enpp1-/-) would exhibit improved insulin signalling and glucose metabolism.

Enpp1-/- mice had reduced body mass compared to wild-type (WT) controls at 16wks (13%; P<0.05) that was likely accounted for by lower muscle mass (Quadratus femoris reduced by 12%; in Enpp1-/- mice; P<0.01). The loss of muscle mass is a likely consequence of the arthritis these mice exhibit.

Under normal dietary conditions Enpp1-/- mice exhibited normal glucose homeostasis with a reduced peak endogenous insulin response, indicating insulin sensitisation. There was no difference in insulin receptor number, distribution or insulin-stimulated Akt, Erk1/2 or GSK3beta phosphorylation between Enpp1-/- and WT osteoblasts, indicating metabolic effects are independent of bone insulin signalling. The undercarboxylated form of osteocalcin acts as a hormone improving energy expenditure, insulin secretion and insulin sensitivity. Interestingly, Enpp1-/- mice exhibited increased levels of under-carboxylated (119%, P<0.05) and un-carboxylated (156%, P<0.05) serum osteocalcin compared to WT. Further studies are required to establish the mechanisms through which NPP1 regulates osteocalcin carboxylation status in bone.

Enpp1-/- mice showed a pronounced obesity-resistance in response to a chronic high fat diet challenge (reduced gonadal, subcutaneous and mesenteric fat mass; P<0.001) but increased brown fat pad mass (P<0.05). Consistent with reduced adiposity, Enpp1-/- mice showed a trend for improved glucose tolerance (n=7) and significantly improved insulin tolerance (P<0.05) compared to WT.

Enpp1-/- mice are protected from obesity and insulin resistant diabetes. The use of tissue specific inhibition of NPP1 activity may represent a novel therapeutic strategy for treating insulin resistance.

OC4

REGULATION OF SKELETAL AND SOFT TISSUE MINERALISATION BY NPP1 (ECTO-NUCLEOTIDE PYROPHOSPHATASE PHOSPHODIESTERASE)

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Ecto-nucleotide pyrophosphatase/phosphodiesterases (NPPs) hydrolyse nucleotide triphosphates to the corresponding nucleotide monophosphate and the ubiquitous mineralisation inhibitor, pyrophosphate (PPi). This investigation examined the role of NPP1 in bone and soft tissue mineralisation using a mouse model lacking NPP1 (Enpp1-/-). Previous studies demonstrated significant changes in the trabecular bone structure of Enpp1-/- mice. Here, we used microCT to examine the cortical bone changes in detail; excised humerus bones from 8, 15 and 22 week old mice were scanned at 0.9 micrometre resolution. No changes were evident in the cortical bone of 8 week old Enpp1-/- mice. However, significant differences were observed in older animals. Cortical bone volume was increased 28% in 22-week Enpp1-/- mice, whilst cortical porosity was reduced 30% and 60% at 15 and 22 weeks, respectively (p<0.001). This was accompanied by a 13% decrease in pore diameter and an up to 38% increase in inter-pore distance (p<0.05). Conversely, cortical thickness was >33% lower in 15 and 22-week Enpp1-/- mice (p<0.001). Endosteal diameter was increased up to 20% at 15 and 22 weeks (p<0.01), whilst the periosteal diameter was unchanged. Thus, the cortical bone from Enpp1-/- mice was thinner but denser and less porous, with a larger marrow space. Scanning electron microscopy (SEM) revealed a decrease in the size and number of blood vessel channels in the cortical bone as well as a 35% reduction in the mean plan area of osteocyte lacunae. We noted that the number of viable osteocytes isolated from the long bones of Enpp1-/- mice was decreased up to 50% (p<0.01). These animals also displayed widespread ectopic joint calcification; in the knee this was accompanied by 30%, 15% and 15% reductions in epiphyseal trabecular bone volume, thickness and number, respectively (p<0.001); tibial subchondral bone was reduced up to 17% (p<0.05). MicroCT and histological analysis of soft tissues also revealed for the first time calcification of the whisker follicles, ear pinna and trachea of Enpp1-/- mice. This soft tissue calcification worsened as the animals aged. Together, these data highlight the key role of NPP1, and thus PPi in regulating calcification of both skeletal and soft tissues.

OC5

THE INHIBITORY ACTIONS OF ATP AND UTP ON BONE MINERALISATION ARE PARTIALLY MEDIATED BY THE ACTIVITY OF NPP1 (ECTO-NUCLEOTIDE PYROPHOSPHATASE/PHOSPHODIESTERASE 1)

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ATP and UTP (>1micromolar) prevent bone formation in vitro by blocking mineralisation of the collagenous matrix. These inhibitory effects are thought to be mediated by both P2 receptor-dependent (via P2Y2, P2X1 and P2X7 receptors) and independent mechanisms, the latter involving hydrolysis of ATP and UTP by NPP1 (ecto-nucleotidase phosphodiesterase/pyrophosphatase 1) to produce the inhibitor of mineralisation, pyrophosphate. This investigation used the NPP1 (Enpp1-/-) knockout mouse model to assess the contribution of NPP1 to the inhibitory effects of extracellular nucleotides on bone mineralisation. Primary osteoblast cultures were obtained from the calvariae of wildtype (Enpp1+/+) and Enpp1-/- mice by trypsin/collagenase digestion and cultured for up to 28 days in alphaMEM supplemented with 50micrograms/ml ascorbate and 2mM Beta-glycerophosphate. Cultured Enpp1-/- calvarial osteoblasts displayed <70% increase in bone mineralisation compared to wild-types (p<0.001). Cell viability and number were unaffected by NPP1 deletion. In Enpp1+/+ osteoblasts, ATP inhibited bone mineralisation by 50% and 80% at 10micromolar and 100micromolar, respectively. In Enpp1-/-cells, the effects of ATP were 10 times less potent, with inhibition of mineralisation only evident at 100micromolar (p<0.001). Since the primary source of ATP in bone is likely to be controlled release from osteoblasts and osteocytes, we also examined whether NPP1 deletion affected this process. Enpp1-/- osteoblasts at all stages of culture (proliferating, differentiating and mature, bone-forming) showed <70% reduction in constitutive release of ATP (p<0.01); this was accompanied by a ~3-fold increase in total intracellular ATP levels (p<0.001). Fluid flow stimulated ATP release up to 8-fold in differentiating and mature Enpp1+/+ osteoblasts; this response was impaired by ~60% in Enpp1-/- cells (p<0.001). Interestingly, when 1micromolar ATP was added to Enpp1-/- osteoblasts the rate of breakdown was unchanged. This may reflect a compensatory upregulation of NPP2, NPP3 and NTPdase 3 since increased mRNA expression (p<0.01) of these ecto-nucleotidases was observed in Enpp1-/- osteoblasts. Taken together, these data confirm that the inhibitory effects of ATP on bone mineralisation are not solely mediated by P2 receptor signalling and that the hydrolysis of ATP by NPP1 to produce pyrophosphate is an important contributing factor.

OC6

HIGH IMPACT EXERCISE SUBSTANTIALLY INCREASED THICKNESS OF THE SUPERIOR FEMORAL NECK CORTEX IN OLDER MEN, DESPITE ONLY MODEST CHANGES IN FEMORAL NECK AREAL BONE MINERAL DENSITY

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BACKGROUND: Studies using 3-D imaging methods have shown that cortical thinning at the superior femoral neck predicts hip fracture in older adults better than femoral neck areal bone mineral density (aBMD). Interventions that increase cortical thickness in this region may improve proximal femur strength to a greater extent than aBMD alone. This study evaluated the influence of high impact exercise on regional femoral neck cortical thickness and femoral neck aBMD in older men.

METHODS: Fifty, healthy community-dwelling older men were prescribed a 12-month high impact unilateral exercise intervention, which progressed to 50 daily multi-directional hops on one randomly allocated leg. Multi-slice quantitative computed tomography (QCT) and dual-energy X-ray absorptiometry (DXA) scans of the hips were performed at baseline and after 12-months of exercise by observers blind to the leg allocation. Regional QCT analysis of the mid femoral neck was used to examine cortical thickness in superior and inferior quadrants. Two-way repeated measures ANOVA was used to identify leg (exercise leg [EL] vs. control leg [CL]) x time (pre vs. post) interactions.

RESULTS: Thirty-five men (mean ± SD, age 69.9 ± 4.0 yrs, height 175.3 ± 6.3 cm, body mass 80.4 ± 8.4 kg, BMI 26 ± 2 kg/m2) exercised for 12 months, attending 91 ± 9% (306 ± 31 sessions completed out of 336 prescribed sessions). Femoral neck aBMD increased in the EL (+0.7%) compared to the CL (-0.9%); ANOVA interaction P=0.003. Cortical thickness in the superoposterior and superoanterior regions increased more in the EL (+52% and +15%) compared to the CL (+17.2% and +4.8% respectively); ANOVA interaction 0.001<P<0.016. Similarly, cortical thickness in the inferoposterior region increased in the EL (+4.8%) relative to the CL (-0.4%); ANOVA interaction P=0.014, although the response in cortical thickness at the inferoanterior region did not differ between legs; ANOVA interaction, P=0.090.

CONCLUSION: Brief high impact exercise considerably increased thickness of the superior femoral neck cortex, whilst only producing modest increases in femoral neck aBMD. Exercise could produce substantially greater increases in bone strength than would be expected from aBMD alone, by targeting adaptation at areas of focal weakness.

OC7

A GWAS IN AN EXTREME HIGH BONE MASS POPULATION SHOWS EXCESS SIGNAL FROM GENES ASSOCIATED WITH BMD IN THE NORMAL POPULATION

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Extreme high bone mass (HBM) may be monogenic (e.g. explained by SOST or LRP5 mutations), oligogenic or polygenic, and may be due to variants in the same genes determining bone mineral density (BMD) as found in the general population. We aimed to determine the extent to which variation in 56 established BMD-associated loci cause raised BMD in an extreme UK-based HBM population(1).

258 unexplained HBM cases (defined as L1 Z-score>+3.2 plus total hip Z-score>+1.2, or total hip Z-score>+3.2 and L1 Z-score>+1.2) were recruited from 15 UK centres, by screening 335,115 DXA scans(2). Individuals with established SOST and LRP5 mutations were excluded by Sanger sequencing (n=3). We performed a GWAS for HBM, genotyping 240 HBM cases using Infinium OmniExpress-12v1.0 DNA analysis beadchips and clustering using GenomeStudio software (Illumina). Controls constituted two previously genotyped populations: (i) unselected (n=5667, 1958 British Birth Cohort [BBC]), (ii) ethnically-matched low BMD (n=900, Anglo-Australasian Osteoporosis Genetics Consortium(3) (AOGC) post-menopausal women with BMD Z-scores -4.0 to -1.5). Samples were assessed for cryptic relatedness, excess heterozygosity/missingness. SNPs with MAF<1%, and/or not in HWE were removed, leaving 181,323 SNPs. The dataset was imputed using the 1000 Genomes Project; SNPs with r2 threshold>0.8 were retained. SNPs were tested for association with HBM using PLINK, assessed separately for each control group.

Results demonstrated over-representation of associations with BMD loci identified from the normal population1 (Figure.1 [HBM vs. unselected BBC], Figure.2 [HBM vs. low AOGC]). Over-representation was greater when HBM was compared against the extreme low BMD population than when analysed against the unselected population, despite the larger population used in the latter analysis. Strongest associations were seen for loci 3p22.1 (CTNNB1 coding for Beta-catenin) OR 1.68 (95%CI 1.55, 1.80) p=7.8x10-7 and 13q14.11 (TNFSF11 coding for RANKL) OR 1.56 (1.43, 1.68) p=3.2x10-5.

Figure 1. Chi-squared Q-Q plot for HBM cases vs. Unselected controls (1958 British Birth Cohort)

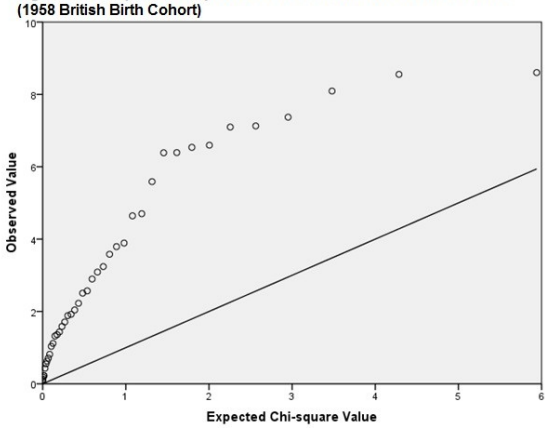
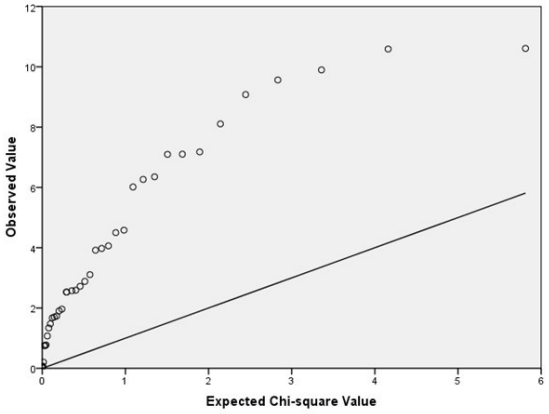


Figure 2. Chi-square Q-Q plot for HBM cases vs. Low BMD controls (AOGC)



Extreme HBM is, at least in part, polygenic in origin, and controlled by the same genes which determine BMD in the general population. Studying extreme populations will enhance the discovery of such genes determining BMD. Whole-exome sequencing of our HBM population is underway to determine the exact variants contributing to HBM.

(1). Estrada Nat.Gen 2012; (2).Gregson OI 2011; (3).Duncan PLoS.Gen 2011

OC8

APPLICATION OF A NOVEL PERFUSION BIOREACTOR WITH INTEGRATED ULTRASOUND STANDING WAVE TRAP FOR AUGMENTATION OF CARTILAGE TISSUE ENGINEERING

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In the absence of effective pharmacological agents and limited success of surgical interventions to treat cartilage defects, application of tissue engineered cartilage grafts is a promising approach to address the problem of cartilage regeneration. However, cartilage grafts generated using conventional tissue engineering strategies are typically characterised by suboptimal cell viability, cartilage formation and mechanical competency, limiting their application in cartilage repair. Ultrasound standing wave trap (USWT), a relatively less exploited non-destructive cell manipulation technique, is capable of spatially

organising cells into levitating 3-D agglomerates. Moreover, stimulation by ultrasound of bovine chondrocytes in 2-D and 3-D cultures in bioreactors is shown to enhance cell proliferation, viability and expression of chondrogenic genes. This presents an exciting opportunity to extend the application of ultrasound to augment conventional cartilage bioengineering strategies.

By utilising a unique multidisciplinary approach, the study aims to bioengineer 3-D scaffold-free neocartilage grafts of human articular chondrocytes (HACs) in custom-built perfusion bioreactors with integrated ultrasound standing wave trap (USWT) to create a dynamic environment conducive for optimal cartilage formation.

The custom-built USWT bioreactor employed a piezoelectric transducer that was fitted on an etched glass capillary. HACs (1×10^6 cells) were introduced into the bioreactors to promote rapid formation of levitating 3-D agglomerates by ultrasonic force fields. The bioreactors were maintained at 37 degrees centigrade in humidified atmosphere and the agglomerates were cultured in chondroinductive media perfused continuously for 21 days. Day 21 explants [~2 mm (diameter) × ~0.15 mm (thickness)] were analysed for cell viability, cartilage formation, expression of cartilage-specific proteins and biomechanical properties.

CellTracker(TM) Green/Ethidium homodimer-1 labelling demonstrated negligible cell death in the explants, indicating no adverse effects from prolonged ultrasonic exposure. The structure of the explants was reminiscent of native articular cartilage and composed of chondrocytes expressing SOX-9 located in lacunae embedded within dense extracellular matrix constituted by proteoglycans and collagen Type II. The elastic modulus of the explants determined by indentation-type atomic force microscopy was comparable to native human articular cartilage.

We have demonstrated the first successful application of USWT in combination with perfusion bioreactor technology for bioengineering robust 3-D neocartilage grafts that are analogous to native articular cartilage.

OC9

MORPHOLOGICAL CHARACTERISTICS OF CHONDROCYTES IN INJURED CARTILAGE CULTURED UNDER VARIOUS CONDITIONS

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In degenerate cartilage, chondrocytes often exhibit increased volume, abnormal morphology, enhanced proliferation/cluster formation and loss of viability [1]. These changes may lead to abnormal matrix production/poor repair, enhancing susceptibility to secondary osteoarthritis (OA). Here, we have developed an in vitro model of mechanically-injured cartilage, which we then cultured in various conditions to study changes in chondrocyte viability and morphology at the injured site.

Full-depth cartilage explants obtained from bovine metacarpal-phalangeal joints, were injured with a single scalpel cut and cultured over 14d in (a) standard-DMEM (Dulbecco modified Eagle medium), (b) 10% fetal calf serum (FCS)-DMEM or (c) 10% synovial fluid (SF)-DMEM (37C,pH 7.4). At various time points, chondrocytes were fluorescently-labelled, examined by confocal microscopy and analysed by Volocity software. Data were from at least n=3 independent experiments at each condition.

After one week of culture, the percentage of dead chondrocytes decreased significantly at the site of injury in explants cultured with FCS-DMEM (1.5±0.4%;P=0.0006) and SF-DMEM (1.3±0.6%;P=0.010) compared to standard-DMEM (20±4%). Chondrocyte volume (which was 711±24cubic-micrometer in uninjured cartilage) increased dramatically close to the injury particularly in FCS-DMEM (1108±29cubic-micrometer;P=0.0002) and SF-DMEM (1229±40cubic-micrometer;P<0.0001) compared to standard-DMEM (978±20cubic-micrometer). In addition, large chondrocyte clusters formed with a total volume of 3839±190cubic-micrometer in FCS-DMEM, and of 4309±478cubic-micrometer in SF-DMEM compared to standard-DMEM (2880±106cubic-micrometer;P<0.001 for both).

After two weeks of culture, chondrocytes near the injury in standard-DMEM were mostly spheroidal. However, in FCS-DMEM and SF-DMEM there were significant morphological changes (P<0.0001;P=0.0013 respectively) which included cellular enlargement, flattening, elongation of cell body and formation of cytoplasmic processes.

The decrease in dead cell counts for explants cultured in FCS and SF compared to standard-DMEM could be due to the presence of DNase. In FCS and SF, distinct changes in chondrocyte volume and morphology (clusters/processes) adjacent to injury may arise from enhanced access of growth/proliferative factors. The increased prevalence of abnormal chondrocytes in the superficial zone along the injury with FCS and SF suggests that the penetration and subsequent action of e.g. growth factors on chondrocytes is a more potent controller of cell morphology than the strength/damage to matrix.

[1] Bush, P.G. & Hall, A.C. (2003). Osteoarth. Cart. 11:242-251.

OC10

PROTECTION OF ARTICULAR CARTILAGE IN VIVO AGAINST SCALPEL-INDUCED INJURY BY A HYPEROSMOLAR SOLUTION

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Introduction

Articular cartilage can be exposed to various injuries during routine orthopaedic/arthroscopic procedures. Cartilage injury can result in progressive cartilage degeneration and development of posttraumatic osteoarthritis. Normal saline, the most commonly-used orthopaedic irrigation solution, has been shown to be suboptimal, however increasing the osmolarity of the solution markedly reduced chondrocyte death in an ex vivo model of scalpel injury (1). Here we studied the effect of a hyperosmolar solution on chondrocyte viability in a reproducible in vivo model of cartilage injury.

Materials and methods

Cartilage injury was induced in the patellar groove of 8 week old male rats by a single pass of a number 11 scalpel blade in the presence of either normal saline (300 mOsm) or hyperosmolar solution (600 mOsm). The animals were sacrificed at 0, 1 and 7 days after the injury. The reproducibility of the injury was assessed by histology and the percentage of cell death analysed and compared using confocal laser scanning microscopy.

Results

The scalpel blade reproducibly induced a partial thickness cartilage defect extending to 58.4 ± 6.8 % of cartilage thickness. The depth and the width of the defect were 202.2 ± 40.5 micrometre and 24.9 ± 1.1 micrometre respectively (n=10; mean ± SEM). A significant decrease in the percentage of cell death around the injured cartilage edge was observed immediately in joints exposed to hyperosmolar solution (8.3 ± 3.4%) compared to normal saline (15.9 ± 6.4%) ($P < 0.01$, paired t-test, n=10). In subsequent time points, chondrocyte death was markedly reduced in joints irrigated with hyperosmolar solution (2.9 ± 0.9 % and 3.9 ± 1.7 % at 1 and 7 days respectively) compared to normal saline (7.4 ± 1.5 % and 7.9 ± 1.4 % at 1 and 7 days respectively).

Conclusion

Increasing the osmolarity of the normal saline markedly reduced chondrocyte death associated with scalpel-induced injury. This irrigation solution might be of clinical value in decreasing the risk of posttraumatic osteoarthritis development after injury and/or promote joint surface repair.

This work is funded by Arthritis Research UK.

(1) Amin AK et al. J Bone Joint Surg Br. (2011) 93(2):277-84

OC11

STATIC AND DYNAMIC EFFECTS ON ARTICULAR CARTILAGE STUDIED WITH A NOVEL ISOLATED JOINT ORGAN CULTURE MODEL

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Disorders affecting articular cartilage are amongst the most common problems in orthopaedics. Various models e.g. cartilage explants and in vivo animal experiments, are available, but the range of variables can be difficult to control. Here, we describe a relatively novel isolated joint organ culture model to evaluate the effect of static and dynamic loads on cartilage.

Twelve bovine metatarsophalangeal joints were isolated and cultured. Six joints were driven by a custom-made machine set to mimic physiological loading patterns, i.e. 30 mins of movement with 30 mins of rest for 12 times per day. The other joints were cultured under identical conditions in the absence of load. Cartilage samples were taken every 7 days for a total of 28 days. Chondrocyte viability in axial and coronal views was determined by fluorescent dyes for living and dead cells and confocal microscopy. Sulphated glycosaminoglycan (sGAG) in the extracellular matrix was quantified by modified dimethyl-methylene-blue (DMMB) spectrophotometry.

Chondrocyte viability visualized in axial views in dynamic models showed 97.9+/-0.5% of the cells were alive by day 28. In coronal views 82.3+/-1.7%, 91.8+/-1.1% and 73.2+/-2.5% of the chondrocytes were viable in the superficial, mid and deep zones respectively after 4 weeks culture. Compared to the viability in static models, a statistically significant improvement in chondrocyte viability in the middle and deep zones was found in the dynamic models after day 7 ($p=0.036$ in middle zone/day 7, $p=0.001$ in deep zone/day 7). Results of sGAG in the dynamic models revealed initial levels were maintained consistently throughout the whole culturing period. However, in static models, sGAG levels dropped 19.5% during week 1, and then remained stable without further change until day 28.

The results supported the validity of this model and demonstrated that the chondrocytes subjected to static or dynamic loads survived well over 4 weeks. Of importance was the finding that the loss of chondrocyte viability and sGAG was less when the joints were subjected to dynamic compared to static load. This whole joint model could be of value in determining the signaling mechanisms which control the response of chondrocytes to dynamic load.

OC12

FACTORS AFFECTING THE EXTENT OF CHONDROCYTE DEATH DURING CARTILAGE DRILLING

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Osteochondral fragments are normally reattached by drilling and insertion of intra-articular screws. Previous studies have shown that drilling causes a zone of cell death (ZCD) around the hole [1]. Here, we have measured the temperature changes of the drill bit and in the cartilage around the hole while drilling under different irrigation conditions.

Holes were drilled into metatarsophalangeal joints of 3-year-old cows using 1.5mm orthopaedic drill bits. Irrigation was either absent, or delivered in copious amounts using normal (0.9%) saline, 20mM calcium chloride saline or 20mM magnesium chloride saline. Osteochondral explants were harvested by chisel and hammer, and placed in 5-chloromethylfluorescein-diacetate (CMFDA) and propidium iodide (PI) to label living or dead cells respectively [1]. Explants were visualised by confocal microscopy and the ZCD quantified. The temperatures of the drilled hole and drill bit were recorded throughout using a calibrated infrared thermal camera.

A ZCD measuring 135+/-15 micrometers was produced following drilling which was reduced to 63+/-3 micrometers using saline irrigation ($n=4; p<0.01$). Calcium chloride saline increased the ZCD to 256+/-22 micrometers compared to the osmotically-balanced control (100+/-16 micrometers; 20mM magnesium chloride saline; $n=4; p<0.01$).

The maximum temperatures measured beside both the drilled hole and at the drill bit were much higher without irrigation. The drilled hole without irrigation was approximately 204 degrees C and this decreased almost to room temperature (26 degrees C) with irrigation. The maximum temperature of the drill bit without irrigation saturated the signal of the thermal camera at approximately 284 degrees C although it was estimated to have reached over 350 degrees C. With irrigation, the maximum drill bit temperature was reduced to 148 degrees C.

Saline irrigation markedly reduced both the temperatures during drilling and chondrocyte death, strongly suggesting that the temperature increase was the major cause of the ZCD. However chondrocyte death was still evident during irrigation, suggesting that the mechanical trauma also caused the ZCD through another mechanism. The increased cell death with calcium chloride saline irrigation indicates that stretch-sensitive ion channels [2] might, in part, be responsible.

[1] Houston DA, etal (2013) Osteoarthritis Cartilage 21(5):721-9.
[2] Mobasheri A, etal (2010) J. Cell. Physiol. 223:511-8.

OC13

ALTERATIONS IN SCLEROSTIN PROTEIN LEVELS IN CHONDROCYTES OF DIFFERENT PHENOTYPES

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Recent work in osteophytes has suggested sclerostin levels may be altered in chondrocytes of different phenotypes. In order to further investigate this we used an in vivo model of altered chondrocyte maturation (equine osteochondrosis) and in vitro chondrocyte culture in monolayer and three dimensions to further investigate sclerostin levels in chondrocytes of different phenotypes. Equine osteochondrosis (OC) is characterised by retention of a transient chondrocytes and subsequent failure of endochondral ossification (EO).

Osteochondral samples were obtained from the distal femur of 18 horses (9 normal horses, 9 with OC). Sclerostin protein was localised by immunohistochemistry (rabbit polyclonal anti-sclerostin antibody (Abcam, UK)). Western blotting was used to verify antibody specificity. Immunohistochemistry was scored semi-quantitatively and a students t-test was used to determine significance.

Equine chondrocytes were isolated from normal cartilage and cultured in monolayer or in agarose beads. Proteins and RNA were obtained and sclerostin levels determined by Western blotting and densitometry and qPCR.

Western blotting confirmed specificity of the antibody. In normal cartilage, sclerostin immunoreactivity had a total score of 0.88 +/- 0.33 significantly different to the 8.1 +/- 0.33 in OC cartilage. In cultured chondrocytes, sclerostin protein was significantly decreased in cells grown in agarose compared to monolayer (80% reduction). In contrast, the RNA levels significantly increased in cells grown in agarose (4 x increase).

The results indicate that sclerostin levels are altered in chondrocytes of different phenotypes in normal chondrocytes and in disease. Sclerostin is increased in i) in vivo lesions of OC, a naturally occurring disorder of chondrocyte maturation and ii) in monolayer, de-differentiated chondrocytes compared to chondrocytes cultured in three-dimensional agarose beads (a model of a stable differentiated chondrocyte phenotype). Our observations are in agreements with the findings in osteophytes, namely that sclerostin is increased in less mature chondrocytes. Finally we also observed that sclerostin mRNA levels were not correlated with protein levels, as reported by a number of other authors previously.

These results provide evidence that sclerostin plays a role other than that of inhibiting bone formation in musculo-skeletal tissue; studies are ongoing to identify that role in chondrocyte differentiation.

OC14

ANALYSIS OF PROXIMAL FEMUR SYMMETRY USING STATISTICAL SHAPE MODELS BASED ON DATA FROM THE OSTEOARTHRITIS INITIATIVE

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For hip joint replacement surgery, the shape of the contra-lateral femur often serves as a template for surgical planning. Recent research has shown that the shape of the left and right proximal femur appears to be symmetrical based on conventional hip geometric measurements (e.g. femoral head diameter). However, these measurements reduce shape to a series of linear measurements rather than taking global shape into

account. The objective of this study was to analyse the symmetry of the left and right proximal femur using Statistical Shape Models (SSMs) which allow a quantitative description of global femur morphology.
We recently developed a system to rapidly and accurately segment the proximal femur from AP pelvic radiographs placing 65 points along its contour. We applied this system to fully automatically generate an SSM of the left and right proximal femurs of 1258 Caucasian females (mean age: 61.3 SD=9.0) without prior diagnosis of hip osteoarthritis. We used the automatically derived SSM to analyse femur shape variation between contra-lateral hips after accounting for shape variation due to positioning. Data for these analyses are from the OAI public use data set(s).
The analysis of global femur morphology based on the generated SSM showed that the average difference between left and right proximal femur shape was within 1.0mm (mean point-to-curve distance) which is small compared to the overall shape variation in the population. Using the obtained segmentation to automatically calculate conventional hip geometric measurements, the average percent asymmetry was 1.5 [SD=1.3] for the head diameter, 2.6 [SD=2.1] for the neck width, 3.1 [SD=2.3] for the shaft width and 2.3 [SD=1.9] for the neck-shaft angle. The average absolute difference was within 1.1mm/2.9degrees for all measurements. This is consistent with previously published results.
Our findings show that the global shape of the left and right proximal femur is highly symmetric without isolated locations of asymmetry. This study also demonstrates that our segmentation system is a time-efficient and effective way to analyse global shape variation across large datasets, having implications not only for orthopaedic surgery planning but also for large scale analyses of bone shape variation and related diseases.

OC15

THE INNATE IMMUNE RESPONSE - A POTENTIAL THERAPEUTIC TARGET IN THE ACCELERATION OF FRACTURE REPAIR IN NORMAL AND OSTEOPOROTIC FRACTURES

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Background:
Osteoporotic fractures represent an enormous unmet clinical need. Inflammation is the earliest response following trauma and initiates a cascade of downstream events crucial for healing of wounded tissues. We have previously found that local administration of recombinant TNF accelerates fracture repair in a murine tibial fracture model (Glass et al PNAS 2011). Here, we investigated the mechanistic pathways and assessed whether this treatment pertains to osteoporotic fractures.
Methods:
1) Tibial fracture model with internal fixation in normal and oophorectomised C57BL/6 female mice. Fracture repair was assessed by CT scanning, immunohistochemistry and in-situ hybridization.
2) Murine air-pouch model: fracture supernatant, generated by incubation of fracture fragments in media to simulate the in vivo cytokine fracture environment, was injected into a murine air-pouch and the cellular infiltrate was assessed at 4h.
Results
* TNF is predominantly expressed by polymorphonuclear cells in the first 72h followed by F4/80+ monocytic cells.
* Local rTNF at the fracture site is only able to accelerate fracture repair if given within 24h; therapeutic dose 0.01ng to 1ng. Conversely, anti-TNF or IL-10 treatment impaired fracture repair.
* Inhibition of neutrophil function resulted in impaired fracture repair.
* Addition of rTNF to murine air-pouch enhanced neutrophil recruitment and promotes the recruitment of monocytes through CCL2 production.
* Using a murine model of fragility fractures, we found that TNF treatment accelerated fracture repair by 40%.
Conclusions:
Fracture healing involves a complex cascade of events involving numerous cell types and the spatially and temporally coordinated release of chemokines, cytokines and growth factors. Our data show that PMNs present during early innate inflammation represent a key cell population that orchestrates the next stage of resolution and regeneration through the recruitment of monocytes. Mechanistically, our data suggest that local administration of a low dose rTNF at the fracture site shortly after injury acts at multiple levels. First it potentiates the early innate immune response to accelerate physiological healing; secondly it promotes the recruitment and osteogenic differentiation of mesenchymal stromal cells. We have thus identified the potential of enhancing the early innate immune response to accelerate fracture repair, including in osteoporotic bone.

OC16

A ONE YEAR IN VIVO STUDY OF THE INFLAMMATORY RESPONSE TO A NOVEL BIORESORBABLE COMPOSITE PLATE FOR THE FIXATION OF BONE FRACTURES

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Background: Conventional bioresorbable polymers have insufficient mechanical properties for high load bearing applications such as fracture fixation. Newer phosphate-based glass fibre reinforced polylactic acid (PBG-PL) composites can achieve mechanical properties similar to cortical bone while retaining biodegradability. However, the in vivo biocompatibility of these novel composites in terms of minimising inflammation and bone resorption secondary to stress shielding or osteolysis is unproven.
Aim: To investigate the in vivo inflammatory response and structural bone changes induced by implantation of a PBG-PL composite plate in an animal model up to 1 year from implantation.
Methods: The PBG-PL composite plate was fixed to the intact right tibia of 15 rabbits which were sacrificed at five time points from implantation (2 to 52 weeks). Histological slides from the plated and contralateral control tibiae were stained with Fast Green, TRAP and Giemsa stains. Macrophage and neutrophil counts as well as structural bone indices (cortical cross-sectional area, cortical thickness, bone resorption) were assessed in a fixed region of interest in the plated and control bones at each of the time points.
Results: Inflammatory cell counts were low in both plated and control specimens (no significant difference) and there was no relationship between the number of inflammatory cells and the duration of implantation. The plated tibia demonstrated a progressive decrease in the cortical thickness (1294.8 micrometre at 2 weeks to 637.9 micrometre at 52 weeks) and a progressive increase in bone resorption beneath the plate as the duration of implantation increased. However this bone loss was offset by the formation of new bone around the plate (Figure 1). Consequently, the cortical cross sectional area was greater in the plated bones compared to the controls (Plated 34.8 mm2 vs Control 25.8 mm2 at 12 weeks) but this difference attenuated as the duration of implantation increased.
Discussion and Conclusion: PBG-PL composite plates elicit minimal in vivo inflammatory response at the site of application up to one year from implantation. Reactive structural changes in the plated bone are likely to represent an adaptive response induced by changes in the mechanical environment rather than osteolysis secondary to inflammation.

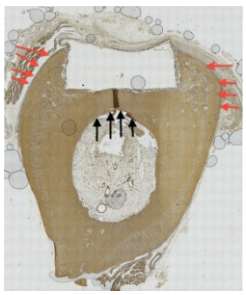

Figure 1

OC17

ROLE OF PERIVASCULAR STEM CELLS IN THE PREVENTION OF ATROPHIC NONUNION

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Introduction

Atrophic non-union is attributed to biological failure of the fracture repair process. It has been reported that perivascular stem cells [(PSCs) also known as pericytes] may serve as a promising source of bone progenitors. Pericyte cells are capable of osteogenesis, chondrogenesis and adipogenesis. They also can provide the trophic factors required for the fracture healing process. The aim of this study was to evaluate in animal model whether PSCs could prevent atrophic non-union forming.

Methods

Perivascular stem cells were isolated from human adipose tissue from 5 individuals using cell sorting according to a previously reported technique. Twelve Wistar rats underwent the procedure to induce an atrophic non-union. The animals were randomly allocated either to the PSC treatment group (n=5) or to the control group (n=7). 5x106 cells were percutaneously injected into the fracture gap 3 weeks after operation. Radiographic parameters, histology, micro-CT and biomechanical tests were used to evaluate the fracture healing outcomes at eight weeks.

Results

At eight weeks, 4/5 animals showed evidence of bone healing with 3/5 showing complete bridging, whereas none of those in control groups had evidence of bone healing. The radiographic parameters showed significant improvement ($p < 0.05$, ANOVA) over the eight-week period in the treatment group. Histology demonstrated bone bridges at the fracture gap in the PSCs treatment group. BMD at callus of the fracture with PSCs injection was significant higher than in control ($p < 0.05$, unpaired t-test). The mean of ultimate force of the callus was 51.0 N (approximately 23.9% of unbroken Tibia).In the control group, fracture deflected immediately without any significant force.

Discussion

The results from this study demonstrate that perivascular stem cells have significant bone regeneration potential in an atrophic non-union model. These cells may have a role in the prevention of atrophic non-union.

OC18

COULD DIFFERENT SHAPES OF NANOSIZED HYDROXYAPATITE HAVE A ROLE IN MODULATING BONE FORMATION?

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Introduction

Bone cells (osteoblasts) produce a collagen-rich matrix called osteoid, which is mineralised extracellularly by nanosized calcium phosphate (CaP). This CaP may be transported by matrix vesicles (MVs) that are released by active osteoblasts. Synthetically-produced CaP nanoparticles (NPs) have great potential for clinical application. However few studies have compared the effect of CaP NPs with different properties, such as shape and aspect ratio, on the survival and behaviour of active bone-producing cells, such as primary human osteoblasts (HOBs). This study aimed to investigate the biocompatibility and stimulatory effects of two differently-shaped hydroxyapatite [Ca10(PO4)6(OH)2] nanoparticles (HA NPs), round- and rice-shaped.

Materials and Methods

The ultrastructural response and initial extracellular matrix (ECM) formation of HOBs to HA NPs were observed using transmission electron microscopy (TEM). A TEM-based X-ray microanalytical technique was used to measure cytoplasmic ion levels, including calcium (Ca), phosphorus (P), sodium (Na) and potassium (K). K/Na ratios were used as a measure of cell viability.

Results and Discussion

Following HA NP stimulation, all measured cytoplasmic ion levels increased. Round-shaped NPs had a greater osteogenic effect on osteoblasts compared to rice-shaped HA NPs. However, they produced only a moderate increase in intracellular Ca and P levels compared to rice-shaped HA NPs. This suggests that particular Ca and P concentrations may be required for, or indicative of, optimal osteoblast activity. Cell viability, as measured by Na and K microanalysis, was best maintained in the round-shaped HA NP group. MV release was observed to increase in HA NP-stimulated HOB cultures (Fig. 1a, no HA NPs, Fig. 1b, HA NPs). Initial formation of osteoblast ECM was altered in the presence of either HA NP. Immuno-TEM identified fibronectin (Fig. 1c, no HA NPs, Fig. 1d, HA NPs) and matrilin-3 as two ECM proteins affected. Matrilin-3 is here described for the first time as being expressed by isolated human osteoblasts.

Conclusion

In summary, this novel and in-depth study has demonstrated that HA NP shape can influence a range of different parameters related to osteoblast viability and activity.

Acknowledgements

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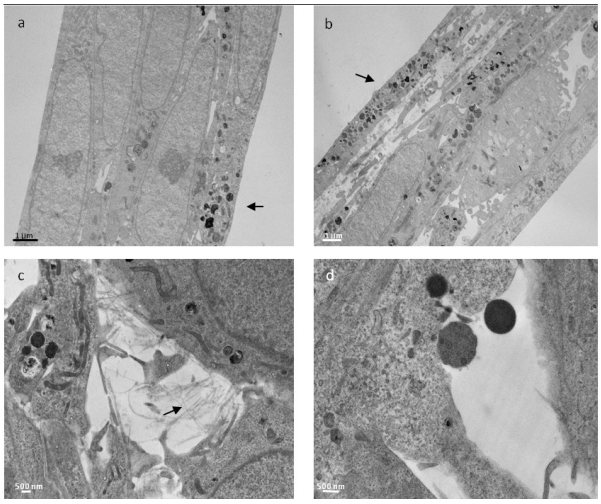

Fig. 1

OC19

THE INFLUENCE OF COMPOSITE SCAFFOLD POROSITY ON CELL DIFFERENTIATION AND ECM PRODUCTION: BONE VS. CARTILAGE

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Introduction

Effective regenerative medicine strategies for articular cartilage damage and osteochondral defects are still in early development. However, by mimicking the architecture and microstructure of the scaffold, it may be possible to control cell response. Our hypothesis was that altering scaffold properties such as porosity would be sufficient to induce different fates and ECM production in cells of the mesenchymal lineage.

Materials and Methods

NanoHA-polymer composites with varying porosities were produced. Nanocomposite structure and mechanical properties were tested. Human MSC-derived osteoblast (MDO) and articular knee chondrocyte (AKC) proliferation were measured. An AKC-MDO co-culture system was also used to mimic the natural niche and to test the possibility of regenerating the bone-cartilage osteochondral interface. Markers of osteogenesis, chondrogenesis and native ECM proteins were measured.

Histological analysis and SEM were carried out to visually evaluate cell response.

Results

Results showed that scaffolds with lower porosity (P60) were best suited for osteoblast growth, whereas scaffolds with higher porosity (P75) were optimal for chondrocyte growth and ECM production. Interestingly, alkaline phosphatase, a marker of osteogenesis, suggested that a marked response was induced in both AKCs that and MDOs cultured on P60 scaffolds. Collagen I propeptide levels were elevated in MDOs cultured on P60 scaffolds. Glycosaminoglycan production by AKCs was higher when cultured on P60 than P75. By contrast, collagen II pro-peptide production, a marker of hyaline cartilage, was elevated following AKC culture on P75 scaffolds. Matrix metalloproteinase-13, which may be a marker of articular cartilage turnover, was elevated in AKCs grown on P75 scaffolds. A live AKC-MDO biphasic construct was developed and maintained on both scaffolds over a 6-week culture period.

Conclusion

We have developed osteochondral scaffolds, which have been shown to induce different cell responses as a result of varying porosity. We now aim to develop a living osteochondral tissue for in vivo implantation, based on a composite biomaterial which possesses the properties of both candidate scaffolds.

Acknowledgements

The authors would like to thank ORUK for the financial support.

OC20

INVESTIGATING THE EFFECTS OF DEMINERALISED BONE MATRIX ON TENDON-BONE HEALING AND AUGMENTATION OF TENDON LENGTH IN AN IN VIVO MODEL.

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Introduction:

Tendon degeneration increases with age and affects tendons at multiple sites, commonly resulting in tears, causing pain and loss of function. Surgical challenges in tendon repair include retraction of the tendon leaving a gap between the tendon and the bone and poor regenerative potential of both tissues. Demineralised bone matrix (DBM) has been shown to be osteoinductive via endochondral ossification and to act as a scaffold for cells in the early stages of tendon healing (Sunder at al 2009). The aim of this study was to investigate tendon repair back to bone using DBM in a model where the length of the natural tendon was compromised.

Method: An ovine patellar-tendon model was used to investigate tendon healing when DBM was used to augment tendon length. Six sheep had surgical release of their patellar tendon, excision of the distal 1cm followed by augmentation with DBM. Animals were subject to gait analysis to determine functional weight bearing (FWB) preoperatively and subsequently at 3, 6 ,9 and 12 weeks post-operation. Animals were euthanized at 12 weeks and histological analysis carried out on the tendon and bone-tendon interface.

Results: After surgery the (FWB) increased at each time point from week 3 until sacrifice at 12 weeks so that at week 12, 78.6% (64.8 to 94.4) load was passing through the operated limb (compared to the pre-operative value). Histological results show that at the DBM-bone interface a direct-type enthesis had formed with graduated regions of fibrocartilage and mineralized fibrocartilage separated by a calcified tidemark. The body of the DBM had remodeled and showed a newly formed crimped collagen structure and numerous tenocytes.

Conclusion: This study showed that DBM could be used to successfully enhance tendon-bone healing as well as augment tendon length. DBM could be used to repair the enthesis in rotator cuff tears (RCTs) where it may reduce the high failure rates currently seen in RCT repairs.

OC21

EFFECT OF METAL IONS AND PARTICLES ON OSTEOBLAST MINERALISATION ON GRIT BLASTED, TITANIUM COATED AND HYDROXYAPATITE COATED PROSTHESIS SURFACES

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Successful osseo-integration is critical for survival of hip resurfacing acetabular components. However, failure of osseo-integration is a common mechanism of prosthesis failure. We have previously shown that cobalt (Co) and chromium (Cr) ions impair osteoblast survival and function at clinically relevant concentrations, which may explain the observed poor integration of these implants. By studying the effect of different surface coating treatments on osteoblast function and mineralisation in presence of Co and Cr nanoparticles and ions, we provide evidence that prosthesis surface coating modification modulates osteoblast responses to metal debris and may improve survivorship of these prostheses.

Human osteoblast cells (SaOS-2) were cultured on CoCr alloy disks (JRI Orthopaedics Ltd, Sheffield) with grit-blasted (GB), titanium porous-coated (PC) and hydroxyapatite-coated (HA) surfaces. Mineralisation was assessed after 21 days in osteogenic media by measuring percentage area of xylenol orange, a fluorescent label that incorporates into newly mineralised matrix. Osteoblast differentiation was assessed by measuring alkaline phosphatase (ALP) activity after 7 days. Osteoblasts in all experiments were treated with Co and Cr nanoparticles (100 particles each of Co and Cr per cell) or metal ions [1000μg/L of Co(II)and Cr(III)].

Osteoblast mineralisation was 92% of HA surfaces versus 0.8% of GB and 0.2% of PC ($p<0.0001$). Osteoblasts treated with nanoparticles showed no mineralisation on GB or PC surfaces, but 62% of the HA surface was mineralised. Osteoblasts treated with metal ions on HA surfaces had significantly higher mineralisation (75%) compared to those on GB (0.4%) and PC surfaces (0.1%). ALP activity of osteoblasts on HA was 48% lower compared to GB ($p<0.0001$) and 52% lower than PC ($p<0.0001$). ALP activity on HA was also lower by 30% compared to PC when treated with nanoparticles and by 22% with metal ions ($p<0.05$).

We found an increased rate of cellular differentiation, with an early decrease in ALP activity and subsequently higher mineralisation on HA coatings. In conclusion, HA coatings appear to protect osteoblasts from the negative effects of metal debris which will be of significant benefit for osseointegration of hip prosthesis.

OC22

STABILISING CARTILAGE TO PROTECT AGAINST OSTEOARTHRITIS PATHOLOGY: LESSONS FROM LONG BONE DEVELOPMENT

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Increasing evidence implicates the re-initiation of embryonic processes, responsible for long bone development, in osteoarthritic pathology. We aimed to establish the role of the Wnt inhibitor sclerostin and its downstream target MEPE, a mineralisation inhibitor, in the maintenance of the healthy joint, and in the regulation of these endochondral processes in osteoarthritic joints.

Str/ort mouse (spontaneous osteoarthritis) knee joints at advancing osteoarthritis stages and age-matched CBA (control) joints were examined, by: (i) visualising alterations in joint integrity by histology and by standard and synchrotron radiation-based computed microtomography; (ii) affymetrix mouse gene microarray profiling of articular cartilage (AC) samples, and (iii) immunohistochemical labelling of sclerostin and MEPE. We also examined the role of sclerostin by studies in embryonic and postnatal growth-plates (immunolabelling) and in mineralising chondrogenic ATDC5 cells (immunoblotting).

Our microarray studies reveal the ectopic recapitulation of endochondral processes in AC in our Str/ort mouse model of osteoarthritis evidenced by the increased expression of markers including Col10a1, Mmp13, Alpl, Phospho1 and Enpp1. Our

immunolabelling reveals enhanced sclerostin expression at the osteochondral interface, and enhanced MEPE expression predominantly in the AC in the joints of CBA mice and in unaffected regions of Str/ort mouse joints. At advanced osteoarthritis stages, focal suppression of sclerostin and MEPE expression is observed in regions localised to areas of subchondral bone-thickening and compromised AC integrity (visualised by microtomography). Strong expression of sclerostin and MEPE are observed in ossified ligaments, menisci, and in osteophytes emerging by endochondral ossification; all increasing, however, with disease severity. To determine the role of sclerostin in endochondral ossification, we also examined its localisation in growing embryonic skeletal elements. Interestingly, sclerostin expression is observed in proliferating and calcifying hypertrophic chondrocytes, but this was absent in all postnatal chondrocytes. Our results also show increased sclerostin expression, consistent with hypertrophic differentiation, in chondrogenic ATDC5 cells.

Our data reveal changes in expression patterns in osteoarthritic mouse joints consistent with pivotal roles for sclerostin and MEPE in restricting the endochondral processes observed in osteoarthritis bone pathology. Further investigation into their underpinning mechanisms will identify whether their targeted delivery can protect against pathological ossification in osteoarthritis.

OC23

PREVENTING INFECTION AROUND OSSEOINTEGRATED TRANSCUTANEOUS AMPUTATION PROSTHESES USING ELECTROCHEMICALLY DEPOSITED HYDROXYAPATITE AND SILVER

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Background

Osseointegrated transcutaneous amputation prostheses avoid problems associated with socket prostheses such as poor function, repeated fittings and pressure sores. The main complication associated with transcutaneous implants is infection. In order to avoid infection soft tissue cells must win the 'race for the surface' against bacteria. Osseointegrated transcutaneous amputation prostheses currently in clinical use are coated with hydroxyapatite (HA) in order to promote fibroblast/soft tissue integration. Silver (Ag) has a broad spectrum of antimicrobial activity and when electrochemically co-deposited with HA, the resultant coating is pure, crystalline and uniform[1].

The aim of this study is to assess fibroblast viability and bacterial colonisation on co-deposited HA and Ag (HAAg).

Methods

10x3mm titanium alloy discs were used. The following surfaces were tested: HA, HAAg, and HAAg that had been preconditioned for 24hours in fetal calf serum (HAAgP24). A 0.13M solution of calcium phosphate monobasic containing silver nitrate (100mg/litre) was used. A current of 200mA for 10 minutes was applied. Silver content was assessed using Energy Dispersive X-ray analysis. Human dermal fibroblast viability and metabolism were assessed using a live:dead assay(n=3) and the Alamar blue assay(n=6) respectively. Surfaces were challenged with Staphylococcus aureus ATCC29213. Direct colony counts (n=3) of planktonic and bacteria in an attached biofilm were performed. SPSS 17.0 was used for statistical analyses.

Results

The median atomic percentage of HAAg was 0.66%. HAAg surfaces were cytotoxic to fibroblasts compared to HA; however, HAAgP24 surfaces were cytocompatible. There was no difference in viability on HAAgP24 compared to HA ($p>0.05$). There was significantly less cell metabolism on HAAg surfaces that had not been preconditioned than on HA($p<0.05$). However, HAAgP24 surfaces resulted in increased cell metabolism ($p<0.05$) and there was no difference in cell metabolism between HAAgP24 and HA ($p>0.05$). HAAg and HAAgP24 reduced bacterial numbers within both biofilms and planktonic suspensions significantly compared to HA.

Conclusion

Both HAAg and HAAgP24 significantly reduced Staphylococcus aureus colonisation compared to HA. HAAgP24 is cytocompatible. This indicates that after 24 hours preconditioning (or clinically 24 hours after implantation), fibroblasts win the race for the surface against staphylococcus aureus on HAAg.

[1]Ghani Y et al J Orthop Res 2012;30:356-63

OC24

IN VIVO ASSESSMENT OF POROUS IMPLANTS FUNCTIONALISED WITH RGD PEPTIDES IN ENHANCING OSSEOINTEGRATED AMPUTATION PROSTHESIS

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Current clinical options are limited in restoring function to amputees, and are associated with contact dermatitis and infection at the stump-socket interface. Osseointegrated Amputation Prosthesis attempts to solve issues at the stump-socket interface by directly transferring axial load to the prosthesis, via a skin-penetrating abutment. However, development is needed to achieve a seal at the skin-implant interface to limit infection. Fibronectin, an Extracellular Matrix protein, binds to integrins during wound healing, with the RGD tripeptide being part of the recognition sequence for its integrin binding domain. In vitro work has found silanization of RGD to polished titanium discs up regulates fibroblast attachment compared to polished control. Electron Beam Melting can produce porous titanium implants which may encourage tissue attachment. This study aims to test whether a combination of biological RGD coatings and porous metal manufacturing techniques can encourage the formation of a seal at the skin-implant interface.

We developed four different augmented transcutaneous devices: Porous, Porous RGD coated, Drilled and Drilled RGD coated. These were implanted in tibial transcutaneous ovine model, n=6, for a period of 6 months. Following explantation we performed hard grade resin histology to assess soft tissue attachment at the transcutaneous interface.

Histological analysis revealed no statistical difference in epithelial downgrowth and epidermal attachment values between the four augmented devices. There were significant increases ($p<0.05$) in the number of blood vessels and the number of cells in the Porous RGD devices compared with Drilled alone. Both Porous and Porous RGD implant groups observed significant increase ($p<0.05$) in soft tissue infiltration compared with the Drilled implant group.

The use of porous structures and RGD coatings increases tissue ingrowth and revascularisation in ITAP devices despite having no effect on epithelial downgrowth and epidermal attachment in a long term ovine model. There were no detrimental effects in the transcutaneous interface formation observed. These augmentation techniques may prove beneficial in preclinical and clinical developments of transcutaneous osseointegrated devices.

OC25

OESTRADIOL AND DHEA MAY INFLUENCE THE DEVELOPMENT OF OSTEONECROSIS IN ADOLESCENTS BEING TREATED WITH CORTICOSTEROIDS

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VEGF couples angiogenesis and osteogenesis, and interruption to VEGF signalling is implicated in the pathogenesis of corticosteroid-induced osteonecrosis. Osteonecrosis particularly affects adolescents but the relationship between sex steroids, puberty and VEGF signalling in osteocytes has not been previously documented. Oestradiol increases uterine and thyroid VEGF production and we have previously demonstrated that dexamethasone reduces osteocyte VEGF secretion. We hypothesised that sex steroids (oestradiol, testosterone) and a sex steroid precursor (DHEA) may influence osteocyte number and/or VEGF secretion via either nuclear steroid receptors or non-genomic pathways.

MLO-Y4 osteocytes were incubated (24-72 hours) with oestradiol, DHEA or testosterone (10-11 - 10-8M) +/- dexamethasone (10-7 M). Cells were subsequently incubated with oestradiol (10-8M) +/- oestrogen receptor (ER) antagonists (tamoxifen or fulvestrant; both 10-

7 M). Other experiments investigated the effects of DHEA (10-8 M) +/- an aromatase inhibitor (anastrozole; 10-7 M) to block its conversion to oestrogens. Cell numbers (MTS) and VEGF164 isoform secretion (ELISA) were measured. The effect of dexamethasone (10-7 M) on the expression of ER alpha and beta as well as G-coupled protein receptor 30 (GPR30) was also measured (qRTPCR). Results were analysed by one-way ANOVA using SPSS.

Oestradiol (8-13% at 10-11-10-8M; $p<0.001$) and DHEA (12-16% at 10-11-10-9M; $p<0.001$) significantly reduced osteocyte cell number. When cells were incubated with oestradiol and ER antagonists, or DHEA and anastrozole, in combination, these effects were abolished. Both oestradiol (22-24%, $p=0.04$-0.002) and DHEA (43%, $p<0.001$) significantly increased VEGF164 secretion. Co-incubation with ER antagonists or anastrozole did not ameliorate these effects suggesting firstly that oestradiol may act via a non genomic pathway and secondly that DHEA exerts independent activity rather than by conversion to oestradiol. Dexamethasone prevented the increase in VEGF164 secretion caused by oestradiol and caused a 2.3 fold reduction ($p<0.001$) in ER alpha gene expression. No effect was seen on ER beta or GPR30 expression.

This study shows that oestradiol and DHEA modulate osteocyte biology and that some of these effects are via non-genomic pathways. An interaction with dexamethasone has also been revealed. These novel findings may have important implications in the pathogenesis of the high incidence of osteonecrosis seen in adolescents treated with corticosteroids.

OC26

A HISTOMORPHOMETRIC AND BIOMECHANICAL EVALUATION OF THE OSSEOINTEGRATION OF LASER-ETCHED IMPLANTS IN AN OVINE MODEL.

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Background and Objectives: The surface composition of an uncemented implant can significantly affect its bony integration. A laser-etched surface has shown promising results; its altered surface topography is known to increase surface wettability and promote the adhesion of osteoblasts. This study aimed to investigate the osseointegration of laser-etched implants in an ovine model. Our hypothesis was that implants modified using a laser-etched technique (LE), would encourage comparable amounts of bone growth, contact and interfacial strength when compared with hydroxyapatite (HA) coated implants. We further hypothesized that increased amounts of bony integration would be measured adjacent to LE implants when compared with machine-finished (MF) and grit-blasted (GB) implants.

Methods: A total of 48 tapered transcortical pins were implanted into the diaphysis of both the right and left tibia of six sheep. Four experimental groups (LE, HA, MF and GB) were investigated (n=12) and implants remained in vivo for 6 weeks. Bone apposition rates were quantified using fluorochrome bone markers administered on days 21 and 35 following surgery. Interfacial shear strength was assessed using a Zwick pull-out testing machine (n=6) and bone-implant contact (BIC) was quantified following histology and analysis using image analysis techniques. Mann-Whitney U tests were used for statistical analysis where p values < 0.05 were considered significant.

Results: No significant difference in bone apposition rate was found when all groups were compared. The interfacial strength of LE and HA implants were significantly greater when compared with GB ($p=0.032$; $p=0.004$) and MF ($p=0.004$; $p=0.004$) respectively. No significant difference between LE and HA implants was seen. However, significantly increased bone-implant contact was measured adjacent to HA implants when compared with LE and GB implants ($p=0.022$ and $p=0.006$ respectively). No significant difference was seen in BIC when LE and GB implants were compared, however all surface finishes demonstrated significantly increased contact when compared with the MF finish.

Conclusions: LE surface modification demonstrated promising results however further research is needed to determine the optimal surface required to maximize bony integration.

OC27

PREVALENCE OF RADIOGRAPHIC HIP OSTEOARTHRITIS IS INCREASED IN HIGH BONE MASS: A CASE-CONTROL STUDY

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Background:

We have previously shown that joint replacement, particularly hip replacement, is more common in high bone mass (HBM). In this study we aimed to determine whether radiographic hip OA is also more prevalent in HBM compared with unaffected family members and general population controls.

Methods:

HBM cases were recruited from 15 UK centres by systematically screening DXA databases. HBM was defined in index cases as total hip Z-score >+3.2 and L1 Z-score >+1.2, or vice-versa, and in first-degree relatives of index cases as total hip Z-score plus L1 Z-score >+3.2; unaffected relatives were recruited as controls. Pelvic x-rays were performed in participants aged >40 years. Age-stratified random sampling was used to select population controls from the Chingford 1000 women and Hertfordshire cohort studies. All radiographs were assessed for features of OA (Croft score, osteophytes, joint space narrowing (JSN), cysts, sclerosis) by a single observer blinded to case-control status (SH) using an atlas. Intra-observer repeatability for most features was good. Logistic regression, using generalised estimating equations to account for within-person clustering (right/left), adjusted a priori for age, gender and BMI.

Results:

530 HBM hips in 272 cases (mean age 62.9 years, 74% female) and 1703 control hips in 863 controls (mean age 64.8 years, 84% female) were analysed, after excluding poor quality films and hip replacements (n=108). The prevalence of radiographic OA, defined as Croft score 3 or above, was increased in cases compared with controls (20.0% vs. 13.62%), with adjusted OR [95% CI] 1.52 [1.09,2.11], $p=0.02$. Both osteophytes overall (OR 2.12 [1.61,2.79], $p<0.01$) and moderate (grade 2-3) osteophytes (OR 2.39 [1.72,3.33], $p<0.01$) were more prevalent in cases. However, no difference in the prevalence of JSN was seen (OR 0.97 [0.72,1.33], $p=0.87$) although there was a trend towards more moderate (grade 2-3) narrowing in HBM cases. Subchondral sclerosis was also more prevalent in cases (OR 2.78 [1.49,5.18], $p<0.01$).

Conclusions:

An increased prevalence of radiographic hip OA and osteophytosis was observed in HBM cases compared with controls. These findings are in keeping with a positive association between increased BMD and OA, and suggest that OA in HBM has a hypertrophic phenotype.

OC28

WEAR OF A ROTATING HINGE KNEE JOINT

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Hinge knee replacements are used for a number of applications and in some designs a rotating component is used in order to reproduce rotational knee movement. This means that in knees of this generic

design wear can occur either through the bushes associated with the hinge mechanism and through the rotating platform. The question we wished to answer was whether wear in rotating hinge knee replacements is as high as with condylar designs and which component of the knee generated the most wear.
Four rotating hinge knees were tested in a force controlled simulator, using force and motion data specified in ISO/TC150/SC4/N189 for testing knee replacements. Every 1 million cycles up to 10 million cycles, the following measurements were taken: AP and rotational laxity; roughness measurements of the metallic tibial and femoral component bearing surfaces; and weight loss measurements of the UHMWPE plastics (bushes, bumper and tibial bearing) utilising soaked controls. In order to measure the load distribution between the axle and the bumper pad pressure sensitive film was inserted between the pad and the femoral component at the start of the test and after 10 million cycles.
All four knees behaved similarly. At 10 million cycles the knees became more rotationally lax than at the tests start. The average gravimetric wear of the bushes on the medial side was 8.11mg (2.2SD) and on the lateral side the wear was almost the same at 12.83mg (2.14 SD). There was no evidence of delamination wear. The weight loss of the tibial plastic at 10 million cycles is 50.036mg. At 10 million cycles the average combined wear of all the plastic components was 77.76mg (SD =6.69) with the tibial component accounting for around 64% of the total wear. Load passing through the pad is greater in hyperextension. After 10 million cycles there is more load passing through the axle than at the start of the test.
This is the first study of wear in a rotating hinge knee joint. Overall the use of rotating hinge knee joints represents a low wear implant for patients with severe bone loss around the knee.

OC29

CHILDHOOD BONE SIZE, MINERALISATION AND DENSITY ARE ASSOCIATED WITH METHYLATION STATUS OF THE CDKN2A PROMOTER AT BIRTH

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We used a population based mother-offspring cohort to explore relationships between methylation status of the CDKN2A gene locus in umbilical cords at birth, and bone size and density measured by DXA in childhood.
Based on differentially methylated sites identified as having strong associations with offspring bone mass from a MeDIP-CHIPmethylation array (Agilent) in 19 subjects, we used pyrosequencing to perform in-depth analysis of the methylation status of 9 CpGs within a region of CDKN2A in umbilical cords of 292 children assessed by DXA (Hologic Discovery) at 4 and 6 years old from the Southampton Women's Survey. Appropriate institutional ethics committee approval and participants' informed consent were obtained.
Percentage methylation varied greatly. After taking account of age and sex, there were negative associations between CDKN2A methylation at 5 of 9 CpG sites and bone indices in childhood (all $p<0.05$). At one of these sites, consistently strong negative associations between percentage methylation and offspring whole body BA, BMC and BMD at both 4 and 6 years were observed. Thus for each 1 percentage point increase in CpG methylation, BMC decreased by 1.0g at age 4 years and 1.8g at age 6 years ($p=0.005$ and 0.008 respectively). Adjustment for percentage methylation at RXRA promoter sites, maternal parity, and maternal smoking, triceps skinfolds and physical activity in late pregnancy (all previously associated with offspring bone mass) did not alter these relationships.
We have demonstrated that perinatal methylation status of CpG dinucleotides within the CDKN2A gene locus is negatively associated with bone size, mineral content and areal density in childhood. These findings, if replicated in other cohorts, might suggest a specific role for CDKN2A in skeletal development and the potential for its use as a novel biomarker for later osteoporosis risk.

OC30

NOVEL MICROCT-BASED METHOD TO ASSESS THE 3D STRUCTURE OF CARTILAGE, SUBCHONDRAL BONE AND OSTEOPHYTES SIMULTANEOUSLY IN MURINE OSTEOARTHRITIS

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Subchondral bone (Sb) remodelling, articular cartilage (AC) loss and osteophyte growth are classic hallmarks of osteoarthritis (OA), although their contribution to disease is not fully understood. Murine models of OA have been instrumental to identify key pathways modulating disease and are becoming essential tools for drug target discovery. However, the potential of murine models has been limited by histopathology-based, low throughput. We present a novel microCT-based method to automatically quantify bone and cartilage changes and osteophyte growth simultaneously in murine OA.
OA was induced by destabilization of the medial meniscus (DMM) in right knee joints. 10-week old C57Bl/6J mice were operated and euthanized 1, 2, 4, 8, 12 and 20-weeks post-operatively. Dissected tibiae were imaged by microCT (Skyscan1172, 5um/pixel) before and after incubation in radiopaque contrast agent (phosphotungstic acid). 3D structural analysis was performed by a software (Matlab) developed in our lab, which maps Sb and AC compartments within automatically generated regions placed on the tibial plate load-bearing areas. Whole tibial epiphysis and osteophytes were segmented and their volume was computed.
Automated analysis of the tibial plate load-bearing regions revealed medial Sb plate thickening (+27%) by 2-weeks post-surgery and an increase in trabecular volume fraction (+7.5%) in DMM compared to contralateral (CTRL). Presence or absence of pre-staining with contrast agent did not affect bone analysis. The whole epiphysis volume was enlarged (+6%) in DMM compared to CTRL by 4-weeks post-surgery and medial osteophytes (found only in DMM samples) occupied 3% of such volume. Average cartilage thickness and volume in the tibial load bearing regions were both significantly decreased by 15% in the medial side at 4-weeks post-surgery in DMM compared to CTRL.
Our automated method detected early changes in Sb, AC and osteophytogenesis in the murine DMM model. This was possible thanks to the excellent contrast achieved by our staining procedure, which allowed to develop a robust, automated software for high throughput computation of bone and cartilage parameters in 3D. Our approach could help phenotyping effectively genetically modified mice used for OA drug target discovery and provides accurate 3D structural information for finite element modelling.

OC31

NBQX, AN AMPA/KAINATE GLUTAMATE RECEPTOR ANTAGONIST, ALLEVIATES JOINT PATHOLOGY AND BONE REMODELLING IN ARTHRITIS.

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Background and hypothesis.
Osteoarthritis (OA) and rheumatoid arthritis (RA) patients have increased synovial fluid glutamate concentrations, which affect pain and inflammation via activation of specific glutamate receptors (GluRs). Abnormal joint loading is associated with OA and subchondral bone change is an early feature of arthritis. Since mechanical loading of bone alters glutamatergic signalling, and GluR activation influences osteoblast and osteoclast phenotype, we

hypothesised that AMPA/KA GluRs are expressed in human arthritic joint tissues and that peripheral administration of NBQX (AMPA/KA GluR antagonist), would attenuate joint pathology in antigen-induced arthritis (AIA) in vivo by influencing bone remodelling.
Methods.
Joint degradation and bone remodelling were related to synovial inflammation scores and GluR immunohistochemistry in tibial plateaux samples from three OA patients undergoing total knee replacement (TKR). NBQX was applied to primary human osteoblast cell lines from three TKR patients and mineralisation assessed. NBQX was injected intra-articularly into the affected knees of AIA rats at the time of arthritis induction and joint tissues taken for QRT-PCR, x-ray, magnetic resonance imaging (MRI), histology (Mankin score) and GluR immunohistochemistry after 21 days.
Results.
NBQX prevented mineralization in all cell lines. Human OA tissues showed extensive degradation and remodeling, with abundant GluR (AMPAR2, KA1) expression in areas of bone/cartilage remodeling. AMPAR2 localised to mononuclear bone cells, including osteocytes, and KA1 to osteoclasts and osteoblasts but not osteocytes in remodelling bone. Similarly, in rat AIA, mononuclear cells and TRAP stained osteoclasts in remodelling bone expressed AMPAR2 and KA1. NBQX treatment significantly reduced end-stage joint destruction ($P<0.05$). X-ray and MRI also revealed a smoother articular surface with fewer bone erosions following NBQX treatment in rats. Increased AMPAR3 mRNA expression in AIA rat patella was restored to normal by NBQX, and coincided with increased mRNAs reflecting osteoclast activation (RANKL), bone resorption (Cathepsin K) and bone formation (COL1A1). Cathepsin K and RANKL mRNA levels, and RANKL:OPG ratios, were reduced by NBQX.
Conclusion.
AMPA/KA GluRs are abundantly expressed in human OA and rat AIA joints in areas of bone remodelling. Intra-articular NBQX treatment significantly reduced joint pathology and markers of bone remodelling in AIA, indicating a potential osteo-protective role in arthritis.

OC32

DEVELOPMENT OF NOVEL MODEL SYSTEMS TO REVEAL THE ROLE OF PHOSPHO1 IN THE INITIATION OF SKELETAL MINERALISATION

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Mouse models have revealed a critical role for the intra-vesicular phosphatase, PHOSPHO1, in skeletal mineralisation. The Phospho1-/- mouse presents with osteomalacia and spontaneous fractures. PHOSPHO1 shows substrate specificity towards phosphocholine and phosphoethanolamine present within matrix vesicle membranes. Interestingly, sphingomyelin phosphodiesterase 3 (SMPD3) is a membrane-bound enzyme that cleaves sphingomyelin to generate phosphocholine and ceramide. The osteomalacia observed in the Smpd3-/- mouse may result from altered membrane lipid metabolism. SMPD3 and PHOSPHO1, through their role in the generation and processing of phosphocholine respectively, may function together to liberate inorganic phosphate (Pi), a critical determinant of matrix mineralisation.
The aim of this study was to better understand the association between PHOSPHO1 expression and mineralisation initiation. To do this we have examined cell and organ model systems that are not reliant on exogenous phosphatase substrates such as beta-glycerophosphate (BGP) for mineralisation.
The ability of PHOSPHO1 to initiate matrix mineralisation was confirmed by over-expressing PHOSPHO1 in MC3T3 osteoblast-like cells (clone-24; poorly mineralising & low endogenous PHOSPHO1). Compared with empty-vector transfected control cells, mineralisation (alizarin red staining) was increased in the presence of BGP (84.6%;$p<0.001$) and 1.5mM calcium (93%;$p<0.005$). Indeed, mineralisation in calcium treated cultures was 68% of that seen in those treated with BGP. We next compared gene expression profiles in MC3T3 osteoblast-like cells (clone-14; highly mineralising & high endogenous PHOSPHO1) supplemented with 1.5mM calcium. By 10-days, when mineralisation was profound, Phospho1 expression (RT-qPCR) had increased 150-fold from unmineralised day 0 levels ($p<0.001$). Alp2 and Smpd3 expression were increased 60-fold by day 10 ($p<0.001$). Increased Phospho1 gene expression was confirmed by protein blot.
Further studies assessed the mineralisation capacity of embryonic day 15 murine metatarsals cultured in ascorbic acid (AA) or AA plus BGP supplemented medium. The amount of diaphyseal mineral formed after 7-days under both treatments was comparable, suggesting that this novel BGP-free model may be useful in future PHOSPHO1 investigations.
We have assessed the mineralisation capacity of an osteoblastic cell line and embryonic metatarsals without exogenous phosphatase substrates. We aim to utilise these models to reveal the relationship between PHOSPHO1 and matrix vesicle lipid metabolism in the generation of Pi.

OC33

AUGMENTATION OF OSTEOCLASTOGENESIS IN THE ABSENCE OF THE P2X7 RECEPTOR IN OESTROGEN DEPLETE CONDITIONS IN VITRO IS DEPENDENT UPON PRECURSOR CELL ORIGIN.

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The P2X7 receptor (P2X7R) is known to be involved in both osteoclast formation and apoptosis, and the gene for P2X7R is highly polymorphic with >600 reported single nucleotide polymorphisms. We have previously found an association of loss-of function P2RX7 SNPs and lower LS-BMD in postmenopausal women. Whilst the exact mechanism for this association is unknown, increased bone loss in postmenopausal women is known to be via loss of oestrogen, due to the increased generation, activity and reduced apoptosis of osteoclasts. In this study we provide evidence that the P2X7R in combination with depletion of oestrogen results in further significantly increased osteoclasts formation with an enhanced resorption activity.
Bone marrow (BM) or splenic precursor cells isolated from P2X7R-/- mice were cultured in osteoclastogenic and in oestrogen depleted conditions on dentine. The formation and function of osteoclasts was then measured. P2X7R-/- BM cultures had significantly reduced resorption ability in comparison to P2X7R+/+ cultures (8.59 μm^2 vs 16.20 μm^2 per osteoclast, $p=0.040$) but only slightly more resorbing osteoclasts. Devoid of oestrogen, cells from both P2X7R-/- and P2X7R+/+ mice had increased osteoclasts and resorption. However, the change in resorptive capacity was significantly higher in osteoclasts from P2X7R-/- compared to P2X7R+/+ mice (10 vs 4 fold, $p=0.010$). Compared to the splenic cells of P2X7R+/+ mice, cells from P2X7R-/- mice had significantly higher resorbing osteoclasts (5 vs 13 resorbing osteoclasts, $p=0.010$) and higher resorption but no change in resorption capacity. In oestrogen deplete conditions, despite an overall increase in osteoclast numbers and resorption similar to BM cultures, the fold change in the number of spleen derived osteoclasts was significantly lower (20.3 vs 72.3 fold, $p=0.011$) as was resorption (32 fold vs 396 fold $p=0.0006$) in P2X7R-/- compared to P2X7R+/+ mice.
This data suggests that the increased osteoclast activity *in vitro* following oestrogen deficiency is via enhanced cell numbers with the P2X7R modulating the effect further. The extent of the effect is dependent on the origin of osteoclasts. These results may help explain the recently demonstrated association between accelerated bone loss in postmenopausal women with a reduced or non-functional P2X7R.

OC34

P2X7 RECEPTOR POLYMORPHISMS MODULATE OSTEOBLAST CELL FUNCTIONS

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The P2X7R gene (P2XR7) is highly polymorphic with >600 reported single nucleotide polymorphisms (SNPs) including both loss-of-function (LOF) and gain-of-function (GOF) polymorphisms. We have previously found an association of LOF P2RX7 SNPs and lower LS-BMD, but the exact mechanism is unknown. In this study we provide data to support our hypothesis that the effects of P2XR7 SNP on osteoblast function maybe driving the observed association to LS-BMD in individuals with P2XR7 SNP.

To determine the effect of different P2XR7 SNPs on osteoblast cell function, Te85 osteoblast cells were transfected with P2XR7 wild type (WT) and various SNPs cDNA. P2X7R pore-formation, intracellular calcium levels, cell proliferation, alkaline phosphatase (ALP) activity and in-vitro mineralisation were measured.

Transfected WT or SNPs P2X7R did not function in Te85 cells, unless co-transfected with a naturally occurring truncated isoform, splice variant B (P2X7B). Upon BzATP stimulation, pore formation was only observed in the co-transfected WT and GOF 155Y SNP. Increased intracellular calcium levels, upon BzATP stimulation, were found in all co-transfected cells, compared to the naïve or single P2XR7B transfected cells. Cell proliferation increased by 18% in cells transfected with P2XR7B, co-transfection of WT reduced cell proliferation by 33% whilst P2XR7 SNPs only reduced proliferation by 10-15% (all compared to naïve cells p= P<0.0001). Transfection of P2XR7B significantly decreased the ALP activity by 23% compared to naïve(p=0.0043). Co-transfection of P2X7B and WT significantly increased ALP activity by 36%(p=0.0016); P2X7B and LOF 496A or 568N P2XR7 SNP gave significant decreases of 15%(p=0.0043) and 13%(p=0.0043) respectively. In-vitro mineralisation was significantly increased compared to the naïve cells with co-transfection of P2X7B and WT(p=0.0022), but no significant effect was observed with any of the P2XR7 SNPs.

The results of this study demonstrate that the P2X7B isoform is necessary for P2X7R-mediated osteogenesis in Te85 osteoblasts cells and that P2XR7 SNPs significantly alter osteoblast cell function which may account for the reduced BMD observed in women with P2XR7 polymorphism. This extends our knowledge into the role of the P2X7R in maintaining bone homeostasis, and may, in the future, help identify people at risk of developing osteoporosis.

OC35

LOSS OF P58IPK, THE CELLULAR INHIBITOR OF PKR AND PERK, CAUSES BONE CHANGES AND JOINT DEGENERATION IN MICE..

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Introduction. The protein kinases PKR and PERK are involved in pro-inflammatory cytokine-mediated cartilage degradation in vitro and endoplasmic reticulum stress-induced arthritis, respectively. The aim of this study was to establish whether knockout of P58IPK, an endogenous inhibitor of PKR and PERK, results in knee joint degeneration in vivo.

Methods. Sections of knee joints from P58IPK-null and wild-type mice aged 12-13 and 23-25 months were stained with toluidine blue and joints scored for degenerative changes using the OARSI system. Bone changes were assessed by radiology and high-resolution μCT. Sections were assessed for the presence of phosphorylated PERK by immunohistochemistry.

Results. Knockout mice exhibited significantly narrower tibias (p<0.01) and smaller epiphyses in both the tibia and the femurs (p<0.05). In addition, older knockout mice demonstrated a significant reduction in total volume inside the periosteal envelope of the femurs (p<0.05), a significant reduction in bone volume in both the tibias (p<0.05) and femurs (p<0.01), and a significant reduction in bone volume fraction of the femurs (p<0.05). OARSI scores were increased in medial femoral condyles of 12-13 month old P58IPK null mice (p<0.05) but decreased in the lateral tibial plateau of null mice. In addition, a severe phenotype was observed in a subset of null mice with complete loss of articular cartilage from the medial compartment and heterotopic chondro-osseous tissue formation in the medial joint capsule. These animals exhibited active PERK throughout the knee joint.

Discussion. This study reveals a critical role for P58IPK in maintaining joint integrity, implicating PKR, PERK and ER stress in bony changes underlying the pathogenesis of joint degeneration in vivo.

OC36

OBESITY PROMOTES THE DEVELOPMENT OF MULTIPLE MYELOMA AND THE ASSOCIATED BONE DISEASE IN VIVO

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Multiple myeloma is a fatal haematological malignancy associated with tumour growth within the bone marrow and the development of an osteolytic bone disease. The relationship between obesity and bone biology is complex, however there is increasing evidence to support an association between obesity and myeloma. The cellular and molecular mechanisms that mediate this association remain unknown. To determine whether obesity promotes myeloma development, we have used the well-characterized 5TGM1 murine model of myeloma, where myeloma will only develop when 5TGM1 myeloma cells are inoculated into immunocompetent C57Bl/KaLwRij mice (KaLwRij) mice and not in closely related C57Bl6 mice. The aim of this study was to determine whether (i) whether myeloma-permissive KaLwRij mice were obese and (ii) whether diet-induced obesity can promote myeloma development in otherwise non-permissive C57Bl6 mice. Using body composition analysis, myeloma-permissive KaLwRij mice had a significant increase in body weight, total fat and percentage fat mass as compared with age- and sex-matched C57Bl6 mice (p<0.05). In addition, KaLwRij mice had a significant reduction in trabecular bone volume (p<0.01) and an increase in bone marrow adiposity (p<0.05). Non-permissive C57Bl6 mice were fed a high fat diet (42% calories from fat) or control diet (10% calories from fat) for 4 weeks, at which point a significant increase in percentage body fat was detected. At this point, mice were inoculated with 5TGM1 myeloma cells. The high fat diet promoted myeloma growth and trabecular bone loss (p<0.05). Removal of the diet upon detection of myeloma reduced tumour burden (p<0.05), but had no effect on trabecular bone volume. Taken together, these studies demonstrate that an increase in adiposity creates a permissive host microenvironment for the development of multiple myeloma and the associated osteolytic bone disease.

OC37

EVIDENCE OF A MYELOMA CELL NICHE: DORMANCY IS AN ACQUIRED STATE

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Introduction

Multiple myeloma is a B cell malignancy characterised by the monoclonal proliferation of plasma cells within the bone marrow (BM). Despite continually improving treatments patients eventually relapse. It has been suggested that some myeloma cells evade chemotherapy by residing within protective niches in the BM. We hypothesise myeloma cells reside within specialised niches close to bone and remain in a dormant state until activated. In this study we aimed to establish if all myeloma cells that colonise the BM grow or whether some cells remain in a dormant state. Secondly, to determine if myeloma cell dormancy is restricted to an intrinsic population of cells or if it is an acquired state in vivo.

Methods

5TGM1-eGFP cells were labelled with a long-chain dialkylcarbocyanine membrane probe (to monitor cell proliferation or dormancy) and injected into C57BLkaLwRij mice. Tumour burden was measured by fluorescent activated cell sorting (FACS),

immunohistochemistry and multiphoton microscopy; bone disease was measured by microCT and static histomorphometry. Sub-populations of proliferating or dormant 5TGM1 cells were isolated from in vitro cultures or ex vivo from BM flushes of 5TGM1 tumour-bearing mice by FACS. Cells were then characterized in vitro in osteoblast conditioned media assays or in vivo by injection into C57BLkaLwRij mice.

Findings

In a preclinical model of myeloma, we identified key stages in myeloma disease development and showed that tumour developed from a limited number of 5TGM1 cells. The majority of 5TGM1 cells that located to the BM remained in a dormant state in close proximity to bone. Both proliferating and dormant sub-populations of 5TGM1 cells were successfully isolated by FACS and characterised. In vitro, cells were cultured in osteoblast-conditioned media, and the proliferation of both populations was inhibited. Injection of these cells in vivo showed proportions of dormant cells could be activated to form tumour colonies; and proliferating cells could become dormant.

Conclusions

We have identified, isolated and characterised populations of dormant and proliferating myeloma cells, when cultured in vitro or in vivo both populations behaved in a similar manner. These findings suggest myeloma cell dormancy is an acquired state and targeting such cells in patients may prevent relapse.

OC38

ZOLEDRONIC ACID AFFECTS OSTEOBLASTS IN VIVO WITH POTENTIAL IMPLICATIONS FOR THE BONE METASTASIS NICHE

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Nitrogen-containing bisphosphonates (NBPs) modify osteoclasts but effects on other cells of the bone microenvironment remain to be established. Administration of NBPs prior to tumour cell injection is reported to reduce bone metastasis formation in model systems, mainly through inhibition of bone resorption. Due to their proposed role as part of the bone metastasis niche, it is of great importance to also study the effects of NBPs on osteoblasts. We have carried out a comprehensive investigation of the early effects of a single dose of zoledronic acid (Zol) on the bone microenvironment in vivo, with particular focus on the osteoblast and the possible changes to the putative metastasis niche.

Female, 6-week old immunocompetent balb/c mice were treated with a single, clinically achievable, dose of 100microg/kg Zol or PBS control (n=7/time point and treatment group). Bone integrity, osteoclast and osteoblast activity and number/mm trabecular bone were assessed 1, 3, 5 and 10 days post treatment using microCT, ELISA (TRAP, PINP) and bone histomorphometry, respectively. The effect of Zol on osteoblast number was further validated in a pilot experiment by anti-GFP immunohistochemistry in 6-week old GFP-Ob expressing Mekong mice (pOBCol2.3GFPemd) 5 days post treatment (n=2/group).

As early as 3 days after treatment, animals receiving Zol had significantly higher trabecular bone volume in the distal tibia compared to control (PBS: 15.67% vs. Zol: 18.92%; p=0.0001). Bone volume continued to increase up to day 10. This rapid effect of the drug was also reflected in a significant reduction in osteoblast number/mm trabecular bone on day 3 (PBS: 9.00 vs. ZOL: 3.71; p=0.0070) and PINP levels (PBS: 106.46ng/ml vs. Zol: 40.52ng/ml; p=0.0022) as well as osteoclast number/mm trabecular bone (PBS: 4.97 vs. Zol: 1.20; p=0.0012) and TRAP concentration (PBS: 7.26U/L vs. Zol: 3.22U/L; p=0.0002, all analyses by unpaired t-test). The Zol-induced reduction in osteoblasts was confirmed in Mekong mice by anti-GFP immunohistochemistry, and complementary studies in immunocompromised animals are ongoing.

These data suggest that the reported anti-tumour effects of Zol using preventive treatment protocols may potentially involve a reduction in the osteoblast niche available for tumour cell homing and colonisation, in addition to reduced bone resorption.

OC39

THE NITRIC OXIDE SYNTHASE INHIBITOR L-NAME BLOCKS THE INCREASE IN LIMB PERFUSION INDUCED BY PARATHYROID HORMONE AND REDUCES THE ANABOLIC EFFECT OF PTH ON BONE

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Although the role played by vascular tone in controlling blood flow to bones has received remarkably little attention to date, there is strong evidence that nitric oxide (NO) donors (vasodilators) have significant anabolic effects on bone in humans. Parathyroid hormone (PTH), the only drug approved for the promotion of osteogenesis, is also well-known to be a vasodilator and to stimulate NO production by endothelial cells. The aim of the present study was to investigate whether the potent NO synthase inhibitor L-NAME (NG-nitro-L-arginine methyl ester) might alter the effect of intermittent PTH on bone architecture by blocking its vasodilatory effect.

Male BALB/c mice (16 weeks, n=8 / group) received daily injections of PBS, PTH[1-34] (80ug/kg/day, i.p.), L-NAME (30mg/kg/day, s.c.) or PTH plus L-NAME for 28 days. Hind limb blood perfusion was measured by laser Doppler imaging. Bone architecture in the femur was imaged by micro-CT ex-vivo. Vascular and canalicular perfusion of bones was analysed by confocal microscopy following intravenous injection of procion red dye.

PTH increased lower limb blood flow by 30% within 10 min of injection, an effect sustained over the 20 min recording period, compared to control ($p<0.001$). Co-treatment with L-NAME inhibited the action of PTH on blood flow but L-NAME alone had no effect. These acute effects of PTH on blood flow were found to be reproductible for at least 28 days. Procion red fluorescence in the osteocyte-lacunar canalicular system was increased by 27% in mice treated with PTH compared to control mice. As expected, intermittent PTH treatment for 28 days increased femoral diaphysis cortical bone volume (+20%; $p<0.001$) and trabecular thickness in the secondary spongiosa of the distal femoral metaphysis (+26%; $p<0.001$). Co-treatment with L-NAME decreased the enhancement of the cortical bone volume (-5%, $p<0.05$) and decreased trabecular bone volume fraction (-16%, $p<0.01$) by reducing trabecular number and increasing structural model index.

These results suggest that NO-mediated vasorelaxation contributes to the bone anabolic action of intermittent PTH.

OC40

SHOULD BISPHOSPHONATE THERAPY BE SUSPENDED AT THE TIME OF FRACTURE IN PATIENTS TREATED WITH A DIRECT BONE HEALING STRATEGY?

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INTRODUCTION:

Bisphosphonates delay callus remodelling. With indirect fracture healing osteoclast activity peaks at the phase of callus remodelling. In direct fracture healing osteoclast activity is crucial at the onset for the initiation of tunnelling cutting-cones, and in a rodent model, continuous bisphosphonate therapy has been shown to impair direct fracture healing.

However, despite the prolonged skeletal retention of bisphosphonates,discontinuation of bisphosphonate therapy prior to the point of peak osteoclast activity during indirect fracture healing diminished the negative effects of bisphosphonate on callus remodelling. Therefore, we aimed to determine whether discontinuation of bisphosphonate therapy immediately post fracture will reduce the deleterious effect of bisphosphonates on direct fracture healing.

METHOD:

Twenty aged Sprague-Dawley rats underwent a standardised procedure of tibial osteotomy and compression plate fixation. Ten animals received daily subcutaneous injections of 1μg/kg Ibandronate for 3

weeks (Pre-#) up to the day of osteotomy. The remaining ten animals received daily Ibandronate injections from the day following osteotomy (Post-#). Six weeks later animals were sacrificed. Fracture repair was assessed with mechanical testing, radiographs and histology.
RESULTS:
One animal from the Post-# group was excluded as external callus was seen on radiographs. The mean(±SD)failure stress in 4-point bending test was significantly lower in the Post-# group compared to the Pre-# group[Post-# v. Pre-# = 14.7(±1.44) Nmm-2 v. 38.0(±5.92) Nmm-2, p = 0.001].On contact radiographs the fracture line remained more clearly visible in the Post-# group compared to the Pre-# group. Histology revealed delayed progression to fracture repair in the Post-# group compared to the Pre-# group.
CONCLUSION:
The failure stress obtained in the Pre-# group is significantly greater (p=0.0009) than that obtained in a previous experiment with continuous bisphosphonate treatment before and after fracture which is similar to that observed in the Post-# group in this experiment. Discontinuation of bisphosphonate at the time of fracture reduced the adverse effects of this class of drug on the mechanical strength of direct fracture healing. Thus, despite the long life of bisphosphonates in bone, it would still appear to be beneficial to stop these drugs on the day of fracture if a direct repair technique is being employed.

OC41

MATERNAL 25(OH)-VITAMN D STATUS IN LATE PREGNANCY IS ASSOCIATED WITH OFFSPRING MUSCLE STRENGTH IN EARLY CHILDHOOD

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Background:
Serum 25(OH)-vitamin D [25(OH)D] concentration is known to influence muscle function. Maternal 25(OH)D status during pregnancy has been implicated in the fetal programming of bone and fat mass, but little is known about its role in determining offspring muscle development. We investigated the associations between maternal serum 25(OH)D concentration in pregnancy and offspring muscle mass and strength at 4 years.
Methods:
A prospective mother-offspring birth cohort, the Southampton Women's Survey (Southampton, UK), was studied. Maternal serum 25(OH)D status was measured at 34 weeks gestation. At 4 years, offspring hand-grip strength (Jamar Dynamometer) and body composition by dual energy Xray Absorptiometry (Hologic Discovery) were assessed. Offspring physical activity (PA) was assessed over seven days using accelerometry (Cambridge Neurotechnology Actiheart).
Results:
326 mother-offspring pairs were included. Maternal serum 25(OH)D concentration in late pregnancy was positively associated with offspring height-adjusted hand grip strength (beta=0.12 SD/SD, p=0.02), which persisted after adjustment for a number of maternal confounding factors (including maternal height, pre-pregnancy body mass index, gestational weight gain, walking speed in late pregnancy and smoking status), duration of breastfeeding and child's physical activity at 4 years (beta=0.12 SD/SD, p=0.03). Maternal 25(OH)D was also positively associated with offspring percent lean mass (beta=0.11 SD/SD, p=0.05), but not total lean mass (beta=0.02 SD/SD, p=0.67). This however did not persist after adjustment for confounding factors (beta=0.07 SD/SD, p=0.24).
Conclusions
Maternal 25(OH)D status during pregnancy is positively associated with offspring grip strength at four years. These results are consistent with a role for antenatal 25(OH)-vitamin D exposure in offspring muscle development.

OC42

ENHANCED MINERALISED MATRIX DEPOSITION ON LASER-ETCHED/HYDROTHERMALLY TREATED TITANIUM SURFACES

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Chemical treatments or structural modifications to Titanium (Ti) implants have been shown to enhance osseointegration. In this study we assessed the influence of combining topographical structuring of Ti using laser etching (LE) with chemical modification achieved by hydrothermal treatment with calcium chloride, on mesenchymal stem cell (MSC) morphology and activity in vitro.
Ti sheet was polished to 1200 grit. A lamp pumped solid laser marking machine etched parallel lines across the surface. To hydrothermally treat samples, CaCl2 was added to an autoclave and processed at 200 degrees for 20 hours. Surfaces were seeded with human MSCs and cultured for 24 hours, fixed and vinculin/DNA localised with epifluorescence microscopy, or studied by SEM. MSCs were also cultured for 28 days in osteogenic medium. Alkaline phosphatase (ALP) activity was determined while mineralised matrix deposition was identified by Alizarin red staining.
LE resulted in the creation of parallel shallow channels (width 135 microns, separation 30 microns) formed from overlapping scalloped depressions. Globular microstructures formed at the periphery of these craters from cooled molten metal. Hydrothermal treatment deposited a nanostructured coating on the surface of polished and etched Ti that was confirmed to contain calcium by XPS and XRD. MSCs adhered in equal numbers to all of the surfaces (polished / LE / polished+Ca / LE+Ca) but there were differences in cell morphology. Cells on the LE surfaces had significantly smaller areas and were less round than those on polished surfaces. There was no effect of hydrothermal processing on cell morphology. SEM identified that cells on the LE surfaces preferentially adhered to un-etched substrate but were able to bridge across the channels by interaction with the globular microstructures. Culture of MSCs under osteogenic conditions identified abundant cell growth on all surfaces. Quantification of alkaline phosphatase activity revealed enhanced levels on hydrothermally treated surfaces and alizarin red staining identified increased abundance of mineralised matrix, in particular associated with etched microchannels.
Ti can be laser etched and hydrothermally treated to create surfaces that display a combination of microtopography and calcium containing nanostructures. In vitro, these materials influence MSC morphology, differentiation and the deposition of mineralised matrix.

OC43

VITAMIN D STATUS IN OBESITY: EVALUATION OF FREE 25(OH)D

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Obese individuals have lower circulating 25(OH)D levels than normal weight individuals. The binding proteins vitamin D binding protein (DBP) and albumin are important determinants of vitamin D action, namely free 25(OH)D. Parathyroid hormone (PTH) is increased in response to 25(OH)D deficiency. We aimed to investigate total and free 25(OH)D, DBP and PTH levels in obese individuals.
In September 2012, fasting serum samples were obtained from 99 men and women aged 55 to 75 years. 54 individuals (48% women, 52% men) were obese (BMI >30 kg/m squared)) and 45 (49% women, 51% men) were lean (BMI 18.5-24.9 kg/m squared). Total 25(OH)D was measured by automated immunoassay (Cobas e411, Roche Diagnostics). Free 25(OH)D index was determined using the equation (Free 25OHD = total 25OHD/1+ (6*10 to the power 3 *albumin) + (7*10 to the power 8*DBP)) (Powe et al). PTH was measured by automated immunoassay (IDS-iSYS), albumin by automated immunoassay (Cobas c311, Roche Diagnotics) and DBP by immunoassay (R&D Systems). Standard deviation scores were calculated by standardising the mean difference between lean and obese groups against the standard deviation of the lean group. Independent

samples t-tests were used to determine significant differences ($p<0.05$) between lean and obese groups.

Total 25(OH)D was lower in obesity (mean difference (95% CI) -0.47 (-1.05 to 0.11) SD scores in men, -0.71 (-1.40 to -0.02) in women)). There was no significant difference in free 25(OH)D index between groups. No difference in DBP was observed in obesity (mean difference (95% CI) -0.33 SD scores (-0.98 to 0.32) in men, -0.28 (-0.93 to 0.37) in women). Albumin was significantly lower in obese women (mean difference (95% CI) -0.91 (-1.71 to 0.10) SD scores) but not in obese men (mean difference (95% CI) -0.41 (-0.97 to 0.15) SD scores). No differences in PTH were observed between the groups.

25(OH)D levels are low in obesity and yet there is no increase in PTH. This is most likely because free 25(OH)D is not low and this is because serum albumin, a binding protein for 25(OH)D, is low in obesity.

		Mean (SD) Lean	Mean (SD) Obese	Standardised Difference
Total25(OH)D , nmol/L	Men	71.24 (27.75)	52.53 (19.85)	-0.469**
	Women	68.20 (29.07)	51.36 (29.24)	-0.712**
DBP, µmol/L	Men	2.50 (0.63)	2.21 (0.74)	-0.329
	Women	2.59 (0.86)	2.31 (0.67)	-0.282
Free 25(OH)D index, nmol/L	Men	0.0043 (0.0017)	0.0036 (0.0014)	-0.229
	Women	0.0040 (0.0015)	0.0042 (0.0043)	0.115
Albumin, g/L	Men	50.21 (2.68)	49.28(2.04)	-0.409
	Women	49.64 (2.09)	47.88 (2.97)	-0.909*
PTH, ng/L	Men	69.26 (42.30)	56.72 (27.25)	-0.324
	Women	72.54 (57.71)	70.67 (46.22)	0.227

(*p<0.05, **p<0.01, ***, p<0.001)

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OC44

COMBINING STATISTICS-BASED RAMAN SPECTROSCOPY AND QUANTITATIVE TWO-PHOTON MICROSCOPY FOR ROUTINE NON-INVASIVE OSTEOCHONDRAL IMAGING

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Osteoarthritis is a debilitating disease which is often only relieved by invasive orthopaedic surgery. Earlier diagnosis of cartilage damage and disease may be crucial to preventing longer-term damage. However, current clinical methods lack the sensitivity required to detect subtle and early changes to musculoskeletal tissues. A decisive, non-invasive imaging system could make such early detection possible and provide the detailed chemical and structural information that could aid in earlier intervention. In addition, obtaining such information, which may be more sensitive to tissue chemistry and structure compared to traditional methods of biochemical analysis without extensive tissue processing, could provide crucial data to materials scientists and tissue engineers for the optimisation of cell-constructs systems more akin to native tissue.

Raman spectroscopy was used to provide information on the presence and distribution of chemical species within lapine osteochondral tissue. All four osteochondral zones were identified following rigorous statistical analysis, solving issues with randomisation of cluster analysis of Raman data, which previously have not been addressed. Peak analysis allowed mapping of specific collagen, GAG and mineral-related chemical groups. Two-photon microscopy provided complementary structural information about collagen structure, alignment and type, and for the first time we report herein a description and distribution of osteochondral collagen alignment, based upon tensor ratio data. This rigorous and comprehensive non-invasive imaging system was successfully developed and applied to the detailed characterisation of osteochondral tissue. Such a system may also aid in the development of novel smart" tissue-engineered constructs and strategies for osteochondral repair."

OC45

FUNCTIONALIZED NOVEL SURFACES TO INFLUENCE MESENCHYMAL STEM CELLS

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It is well understood that cell response to their physical environment regulates gene expression and even differentiation. The exploitation of stem cells in tissue engineering and regenerative medicine approaches requires a greater understanding of these mechanisms. This project aims to explore polymer topographies and their region-specific functionalization with biomolecules, as surfaces to influence mesenchymal stem cell (MSC) biology. These studies will help define parameters in scaffold development for musculoskeletal repair.

Centrifugal forces spread thin films of polymers onto a flat substrate by spin coating. Phase separation of contrasting immiscible polymers generates diverse micro-landscapes. Addition of water to this further accentuates the pattern by inducing pores and creators. Solutions of immiscible polystyrene (PS) and poly(methyl methacrylate) (PMMA) solutions were demixed at various ratios (v/v) and spun at speeds > 8,000rpm under humid conditions. Topographical surfaces were imaged using; atomic force microscopy and scanning electron microscopy. Primary adult human MSCs, isolated from bone marrow and characterized by flow cytometry and tri-lineage differentiation, were used to assess the biological response via immunofluorescent and histological staining. Zero length crosslinking was used to immobilize proteins.

After evaluation of a range of demixed ratios/ concentrations/ solvents a selection of surfaces (PS:PMMA [(a)40:60,(b)50:50,(c)60:40] 3%w/v in toluene) were further characterized. These generated opposing raised PMMA and low lying PS islands [(a) 8µm2,(b)8-12µm2,(c)12-15µm2]. Saturated humid conditions induced breath figure patterns generating average crater-like features of (a) 0.5µm,(b)0.7µm,(c)1µm in height. Cells displayed specific interaction with these features, concentrating focal adhesion plaques at these points. Polymer composition alters the dispersion pattern of these crater-like structures, changing cell adhesion profiles, morphology and cytoskeletal organisation.

Cells on 40:60 were shorter triangular and polygonal shaped, compared to elongated multipolar and hyperbolic rectangles on 60:40.

Cell morphology (circularity, area, etc.) was assessed and correlated with polymer surface composition. Immobilization of biomolecules to individual polymer islands on the mixed surfaces allows us to understand how organized display of ligands influences cell activity.

This approach provides greater insight into the effects of chemically defined topographies and how they influence and control MSC activity.

OC46

BMP-9 INDUCES THE CALCIFICATION OF VASCULAR SMOOTH MUSCLE CELLS

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The process of vascular calcification shares many similarities with that of skeletal mineralisation. Whilst, the cellular mechanisms responsible are not fully known, BMP-9 has been shown to exert direct effects on both bone development and vascular function. Therefore, in the present study we have investigated the role of BMP-9 in vascular smooth muscle cell (VSMC) calcification.

Murine VSMCs were cultured in calcifying medium containing Na2HPO4/NaH2PO4 for up to 14d. Calcium deposition was confirmed by alizarin red staining. Calcified VSMCs showed increased Runx2, Bmp2 and PiT-1 mRNA expression ($P<0.001$), which are recognised osteogenic markers of vascular calcification. BMP-9 mRNA expression was significantly up-regulated by 7d (1.4 fold; $P<0.05$) in calcified VSMCs. Furthermore, BMP-9 treatment (50ng/ml) increased VSMC calcium content (3.4 fold; $P<0.05$), ALP activity (10.1 fold; $P<0.001$) and mRNA expression of osteogenic markers ($P<0.001$). BMP-9-induced calcium deposition was significantly reduced (68%; $P<0.001$)

following treatment with the ALP inhibitor 2,5-Dimethoxy-N-(quinolin-3-yl)benzenesulfonamide (3μM) confirming the mediatory role of ALP in this process.
BMP receptor expression, including ALK1, ALK2, BMPR-II, ActR-IIA and ActR-IIB, was detected in mouse VSMCs. The inhibition of ALK1 signalling using a soluble chimeric protein (ALK1-Fc) significantly reduced calcium deposition (85%; $P<0.001$) and ALP activity (33%; $P<0.01$), confirming that BMP-9 is a physiological ALK1 ligand.
Signal transduction studies revealed that BMP-9 (0.5-50ng/ml) induced Smad1/5/8 and Smad2 phosphorylation. As both Smad proteins directly bind to Smad4 it is possible that Smad4 is a central regulator of the BMP-9 triggered response in VSMC calcification. Indeed Smad4-siRNA silencing reduced BMP-9 induced ALP activity (72%; $P<0.001$) and calcium deposition (59%; $P<0.05$).
Vessel calcification in Chronic Kidney Disease begins pre-dialysis, with factors specific to the dialysis milieu triggering accelerated calcification. Intriguingly, BMP-9 was markedly elevated in serum from dialysis patients (234%; $P<0.001$). Whilst no correlation between serum BMP-9 concentration and calcium/phosphate concentration was noted, a significant correlation (0.712; $P<0.05$) was observed between dialysis time and BMP-9 concentration in patients receiving haemodialysis.
These novel data demonstrate that BMP-9 appears to play a critical role in inducing vascular calcification, and may represent a novel potential therapeutic target for clinical intervention.

OC47

ENDOTHELIAL CELLS ACCELERATE THE OSTEOGENIC DIFFERENTIATION OF PERICYTES

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The ability for mesenchymal stem cells (MSCs) to differentiate into osteocytes, chondrocytes and myocytes holds great promise for tissue engineering. MSCs derive from a perivascular niche in vivo where they reside as pericytes. The intimate contact between pericytes and endothelial cells (ECs) suggests the existence of heterotypic cell-cell crosstalk that regulates pericytes in their local microenvironment. Knowledge of mechanisms mediating mesenchymal activation and osteogenic differentiation of pericytes may facilitate therapeutic exploitation of MSCs in vivo where accelerated bone regeneration is desirable. We set out to explore the influence of ECs on the osteogenic potential of pericytes.
Pericytes (CD146+/34-/45-/56-) from human fat and muscle were sorted using fluorescent activated cell sorting. Flow cytometry and PCR were used to confirm purity. Co-expression with MSC markers (CD105 and CD90) and the capacity for bone, fat and cartilage differentiation in vitro was used to confirm MSC phenotype. For two-dimensional cocultures, pericytes were seeded onto a monolayer of ECs (human umbilical vein ECs or adipose ECs). For three-dimensional cocultures, fluorescently labelled ECs and Pericytes were seeded onto a thick layer of Matrigel and observed using time-lapse microscopy. Basement membrane production was assessed using immunohistochemistry.
Two-dimensional cocultures were exposed to osteogenic media for 21 days. The role of EC-released soluble factors on pericyte differentiation was determined using a Transwell co-culture system. Wnt pathway modulators (CHIR, ICG and C59) were used to investigate the role of Wnt signalling this setting.
Sorted pericytes expressed MSC markers and exhibited trilineage differentiation in vitro. Pericytes and ECs integrated, and contributed basement membrane proteins (CollagenIV and Laminin) in two- and three-dimensional cocultures. Pericytes and ECs formed vascular networks in three-dimensions.
The osteogenic differentiation of pericytes was accelerated in the presence of ECs. This effect was independent of direct contact, indicating that the effect occurred through soluble factors. Coculture in the presence of the Wnt modulators confirms that this effect is in part, mediated through Wnt signalling.
Our data suggest that ECs influence the osteogenic differentiation of MSC pre-cursors. Therapeutic targeting of EC-pericyte signalling may enable manipulation of MSC differentiation potential in vivo.

OC48

ATP AND UTP ARE POTENT INHIBITORS OF VASCULAR CALCIFICATION

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The pathological process of vascular calcification shares some similarities to physiological skeletal mineralisation, and involves hydroxyapatite deposition in arteries and cardiac muscle. Whilst vascular calcification has severe clinical consequences, the cellular mechanisms responsible are not fully elucidated. ATP and UTP (>1micromolar) inhibit bone mineralisation via P2 receptor-dependent and independent mechanisms. The latter involves the hydrolysis of extracellular nucleotides by NPP1 (ecto-nucleotidase phosphodiesterase/pyrophosphatase 1) to produce pyrophosphate (PPi), a key mineralisation inhibitor. This study investigated whether extracellular nucleotides also regulate vascular calcification. Vascular smooth muscle cells (VSMCs) were cultured in calcifying medium containing 3mM phosphate for 14 days. Calcium deposition was confirmed by alizarin red staining. Calcified VSMCs showed increased Runx2, Bmp2 and PiT-1 mRNA expression, which are recognised osteogenic markers of vascular calcification. We found that VSMCs express multiple P2 receptors (P2X1-7, P2Y1,2,4,6,12,13,14) and that expression was upregulated in calcifying cells compared to non-calcifying control cells. A key source of extracellular ATP is controlled release from cells; constitutive ATP release from VSMCs was >50% higher in calcifying cells. Removal of this endogenous, locally released ATP by apyrase (an ecto-nucleotidase which hydrolyses ATP to ADP and phosphate) resulted in a 45% increase in VSMC calcification. Culture with exogenous ATP and UTP (>1micromolar) decreased VSMC calcification by <80% and 90%, respectively ($p<0.001$). Additionally, MRS2578 (selective P2Y2 receptor agonist) and Bz-ATP (P2X7 receptor agonist) both inhibited calcification by <70% ($p<0.001$). ADP (P2Y1, P2Y12 and P2Y13 receptor agonist) and UDP (P2Y6 receptor agonist) were without effect. We observed that ATP and UTP increased VSMC total NPP activity by <3-fold ($p<0.001$). Alkaline phosphatase activity, which is very low in VSMC cultures, was also increased 2-fold in ATP and UTP-treated VSMCs ($p<0.001$). Indicating a role for P2 receptor-independent mechanisms in the inhibitory actions of extracellular nucleotides, we found that >1micromolar CTP and GTP (which do not activate P2 receptors but are hydrolysed by NPP1 to produce PPi) inhibited VSMC calcification by ~70%. Furthermore, in NPP1 knockout VSMCs the inhibitory effects of ATP on vascular calcification were 10 times less potent. Combined, these data indicate an important role for extracellular nucleotides in the regulation of vascular calcification.

OC49

CAN SELECTED AND EXPANDED BONE MARROW STROMAL CELLS MAKE NEW BONE :
A RANDOMISED CONTROL STUDY

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Introduction
Regenerating new bone by cell therapy could provide therapeutic options in many conditions such as fracture non-unions and osteo-chondral defect regeneration in advance OA. In this randomized controlled study we evaluated the efficacy of new bone formation by bone marrow derived stromal cells (BMSC) in patients with non-union.

Methods
An ethically approved and adequately powered single centre randomised control trial recruited 35 patients for treatment of non-unions with BMSC. Bone marrow was harvested and culture expanded in autologous serum at our local MHRA-licensed facility (Oscell, Oswestry, UK).
Following selection by adherence and in vitro culture expansion using autologous serum, cells in serum and serum alone was randomised for insertion at one of the two fracture sides by Stratos computer software. Patients and the operating surgeon were blinded to the side of cell insertion. Such method of randomisation created internal controls at the fracture sites- one side receiving the cell-test side and other, not-control.
Serial radiographs extending up to an average of twelve months were evaluated by four independent assessors blinded to side of cell insertion. Callus formation and bridging of fracture was compared for test and control side. Radiological and clinical outcome at final follow-up was also noted.
Results
Thirty five patients were recruited (21 males, 14 females; mean age 51.2+/-13.2SD). The mean duration of non-union was 3+/-2SD years, with a mean 3.5 (range 1-12) surgical interventions prior to BMSC insertion. Five patients had diabetes.
New callus formation and fracture bridging was slow, with no significant difference between the side of cell-insertion and control. Union was achieved in 22 patients. Age at accident, having diabetes and cell doubling time during culture predicted union (r2=0.63, p=0.017) with multivariate analysis.
Conclusion
Our observations have identified three factors in the biology of patients failing to unite. A novel finding is that relatively slow rates of cell division in the laboratory correlated with poor healing. The addition of several million cultured cells did not improve bone formation significantly in this study.

OC50

ENHANCED OSTEOGENIC INDUCTION OF EMBRYONIC BONE DEVELOPMENT BY VASCULAR ENDOTHELIAL GROWTH FACTOR

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Vital for skeletal development and fracture repair is the presence of a functional microvascular network. Vascular endothelial growth factor (VEGF) a potent mediator of angiogenesis plays a critical role in the osteogenesis of the developing bone. Understanding the intercellular signalling and interactive processes of the vasculature in bone development can lead to future therapeutic strategies for the field of bone regenerative medicine. This study has examined the direct role of VEGF in the development of embryonic bone using a novel three-dimensional organotypic culture system.
E11 chick femurs were isolated and placed in an organotypic culture set up in basal culture medium (serum free) or basal culture medium supplemented with VEGF 25ng/ml & 100ng/ml respectively and cultured for 10 days. Organotypic femurs were fixed and analysed by microcomputed tomography (microCT) and assessed histologically for proteoglycans (alcian blue) and collagen (Sirius red) expression and examined immunohistochemically for the endothelial vascular marker CD31.
After 10 days, the femur length (mm) displayed a modest increase from 8.32mm in basal organotypic culture conditions to 8.50mm and 9.41mm in VEGF (25ng/ml) & (100ng/ml) supplemented culture conditions respectively. Microcomputed tomography analysis (10micron resolution) demonstrated a significant increase in microCT parameters in the VEGF stimulated femurs compared to the basal control cultured femurs: Bone Volume (mm3): Basal=0.214±0.067; VEGF(25ng/ml)=0.226±0.052, VEGF(100ng/ml) =0.339±0.042 (**P<0.01); Trabecular Number: Basal=0.180±0.049; VEGF(25ng/ml)=0.243±0.042 VEGF(100ng/ml)=0.342±0.057 (***P<0.001); with reduced Trabecular Spacing: Basal=5.91±1.86; VEGF(25ng/ml)=4.19±0.087 VEGF(100ng/ml)=2.96±0.99 (***P<0.001). However, Trabecular Thickness (mm3) remained the same between all the femur cultured groups.
Increased levels of Sirius red staining indicative of collagen production was observed in the femurs that were cultured in the VEGF additive culture groups compared to basal organotypic cultures. Additionally, a marked increase in the level of cells within the diaphyseal region expressing the vascular marker CD31 was observed.
The current studies demonstrate the direct role of VEGF on osteogenic induction of early embryonic bone development. Understanding the synergistic actions of osteoprogenitor-endothelial interaction; angiogenic and osteogenic growth factors using three dimensional organotypic tissue culture models will underpin and inform the skeletal regenerative process for future therapeutic strategies in solving problems of delayed and non-union bone fracture pathologies.

OP1

POTASSIUM SUPPLEMENTATION AND BONE METABOLISM: A META-ANALYSIS

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Osteoporosis remains a major cause of mortality and morbidity world-wide. The role of acid-base homeostasis as a determinant of bone health, and the potential contribution of fruit and vegetables in promoting skeletal integrity remains a subject of debate.
We have conducted the first-ever meta-analysis to assess the effect of alkaline potassium salts on calcium metabolism and bone health. The objective was to investigate the effects of potassium bicarbonate (KHCO3) and potassium citrate (KCitr), compared with placebo, on urinary calcium and acid excretion, markers of bone turnover and bone mineral density.
A total of 15 studies were eligible for inclusion in the meta-analysis. Analysis was conducted using Review Manager (Version 5; The Cochrane Collaboration). A random effects model was used and results are presented as the mean difference (95% confidence intervals). Authors were contacted to provide missing data from the available publications.
The results show that KCO3 and KCitr markedly lower urinary excretion of calcium, net acid and markers of bone resorption, compared with placebo. For KHCO3, the mean difference (95% CI) in the change in Ca excretion was -4.96 (-7.17, -2.76), P<0.001. For KCitr the mean difference was -0.85 (-1.43, -0.26), P= 0.005. The mean difference in NAE was -49.03 (-62.27, -35.79), P<0.001 and -32.52 (-52.90, -12.14), P=0.002 for KHCO3 and KCitr, respectively. The mean difference in the effect on the bone resorption marker NTX was -7.62 (-14.97, -0.26), P<0.04 for KHCO3, and -4.38 (-5.22, -3.54), P<0.00001 for KCitr. For all markers combined the mean difference was -2.86 (-3.71, -2.02), P<0.00001 and -7.80 (-13.01, -2.59), P=0.003 for KCO3 and KCitr, respectively. There was no effect on bone formation markers. The effect of KCitr on combined formation markers was -1.04 (-2.10, 0.02), P<0.06.
In conclusion, this meta-analysis confirms that administration of alkaline potassium salts leads to significant reduction in renal calcium excretion and acid excretion, compatible with the concept of increased buffering of hydrogen ions by raised circulating bicarbonate. That this neutralisation of dietary acid load has beneficial effects on bone is demonstrated by a clear reduction in bone resorption. Further longer-term bone health studies are now urgently required.

OP2

THE EFFECTS OF METAL-ON-METAL HIP IMPLANTS ON BONE CELL BIOLOGY IN VIVO

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Introduction:
Metal-on-Metal (MoM) hip replacements have been used extensively during the last decade. However, over recent years their use has

significantly declined due to the increased number of adverse local tissue reactions to the metal debris derived from the implant. A number of studies have investigated the effects of MoM implants on the surrounding soft tissue. However, less is known about the effects on bone and the underlying mechanisms. Therefore, the aim of this study was to investigate the effects of MoM implants on bone biology in vivo.

Methods:

Bone samples were collected from MoM patients at the time of revision surgery. The specimens were collected from waste bone from the rim of the acetabulum. Samples were also taken from a similar site in patients undergoing Primary Total Hip Replacements (n=6) which acted as the comparable control group. The bone samples were prepared using the dental techniques of periodontal bone processing and examined by histochemistry and immunocytochemistry to identify cellular changes and localise the presence of RANK-L, OPG and TRAP. Imaging analysis provided semi-quantification data on the immunocytochemical findings. Protein was also extracted from bone samples using a chaotrophic agent (4M guanidinium chloride), separated by SDS polyacrylamide electrophoresis and the presence of RANKL and OPG characterised further by immunodetection using Western blot analysis.

Results

Histochemical analysis identified large areas of adipocytes together with areas of eosinophilic bone trabeculae. Immunohistochemical staining for TRAP, a marker of osteoclast activity, was markedly high in MoM bone tissues. Likewise, there was significantly more staining for RANK-L, an activator of osteoclast development and function, in MoM samples compared to the control ($P<0.01$). Interestingly, there were also higher levels of staining for the osteoclastogenesis inhibitory factor, OPG, in MoM samples compared to control ($P<0.05$). The presence of RANKL and OPG was confirmed following immuno-detection by Western blot analysis.

Conclusion

Our data suggests that metal debris released from MoM implants in vivo affects normal bone homeostasis by altering cellular phenotype and increasing the activation of osteoclasts. These findings may contribute to the observed bone-related complications of these prostheses seen within clinical practice.

OP3

TARGETING ADIPONECTIN INCREASES OSTEOBLAST AND CHONDROCYTE DIFFERENTIATION AND FUNCTION VIA BOTH ADIPONECTIN RECEPTOR-1 AND -2

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There is a significant link between obesity and musculoskeletal disease, however the cellular and molecular mechanism underlying these interactions remain unclear. Certainly the release of adipocyte-derived factors (adipokines) has shown deleterious effects on cells of the bone and joint, suggesting contributory roles in disorders such as osteoporosis and osteoarthritis. However, a number of good adipokines also exist, conferring beneficial systemic effects. These include adiponectin, which is associated with increased lifespan and reduced levels of heart disease and type II diabetes. In addition, we have shown that loss of adiponectin increases tumour burden in a murine model of multiple myeloma, and pharmacological stimulation of adiponectin can increase bone volume and reduce tumour-bone disease.

To investigate the cellular and molecular mechanism through which adiponectin may confer beneficial effects in bone and joint, we utilised murine osteoblast-like 2T3 cells and ATDC5 chondrocyte-like cells to manipulate adiponectin signalling using a combined pharmacological and molecular approach of recombinant adiponectin, the apolipoprotein A mimetic L-4F which increases adiponectin, the adiponectin receptor agonist ADP355 and siRNA knockdown of both adiponectin receptors (AdipoR1, AdipoR2), and assessed in vitro proliferative rates, differentiation, function (mineralization and matrix formation) and gene expression.

Treatment of 2T3 osteoblasts with either adiponectin, L-4F or ADP355 (0.01-100uM) dose dependently and significantly increased cell proliferation to a similar extent. This was associated with an increase in mineralization (up to 25% above control, $p<0.01$) and alkaline phosphatase levels (18% of control, $p<0.05$), and runx2, col1a1, alkaline phosphatase, osteopontin and osteocalcin gene expression. Deletion of AdipoR1, AdipoR2 or both significantly decreased mineralization, runx2 and alkaline phosphatase and blocked the positive effects of adiponectin and related drugs described above.

In addition, adiponectin stimulated ATDC5 chondrocyte proliferation ($p<0.01$) and Col2a1 gene expression. Matrix production was also increased following adiponectin treatment and decreased following siRNA knockdown of AdipoR1, AdipoR2 or both simultaneously, as observed in 2T3 osteoblasts.

These studies suggest that adiponectin and related mimetic drugs promote osteoblast and chondrocyte differentiation and function and may represent a novel target to treat disorders of bone and joint.

OP4

LIPOSOMAL THERAPEUTIC DELIVERY SYSTEM FOR POLYMETHYL METHACRYLATE BONE CEMENT

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Poly(methyl methacrylate) (PMMA) bone cement is a material widely employed in orthopaedics for procedures such as joint arthroplasty. A high number of cemented implants fail at an early stage due to infection, which results in the patient undergoing several complicated surgeries, usually associated with high costs and poor outcomes. To reduce the likelihood of infection, powdered antibiotics are incorporated into the cement to create a local therapeutic release. Commercial antibiotic-loaded cements employ 0.5-1g of gentamicin sulphate, which can agglomerate and weaken the cement. The antibiotic is initially released in an uncontrolled burst from surface agglomerations and sub-inhibitory levels are released thereafter, promoting the formation of resistant bacteria.

A novel coating has been developed which allows liposome vesicles to be dispersed in the liquid component of PMMA bone cement. The dispersion of 100nm liposomes was studied using fluorescence microscopy and transmission electron microscopy. The release of these liposomes with encapsulated gentamicin sulphate from a commercial cement was investigated. The effect of the novel liposomal system on mechanical and fatigue properties and the antimicrobial activity against S. aureus was also studied.

The novel coating enhanced the dispersion of the liposomes in PMMA. As a result of this, a controlled and prolonged release of antibiotic was achieved and the total percentage of antibiotic released surpassed that of commercial antibiotic-loaded cements. The liposomal system also enhanced the antimicrobial performance and crack resistance of the commercial cement.

In conclusion, this technology demonstrated enhanced properties when compared to the powdered antibiotic system employed in commercial cements. Liposomes can deliver multiple therapeutic agents, both hydrophilic and hydrophobic in nature, from a single vesicle. This has the potential to create a synergistic effect, broaden the spectrum of antibacterial activity, prevent resistant microorganisms from developing, reduce dose-related toxicity and enhance bacterial inhibition. Certain phospholipids have also been shown to have calciotropic properties and therefore there is also potential to enhance bone growth using this novel system. This technology has the potential to reduce implant failure, morbidity and the need for costly and complicated revision surgery, which is a burden on both healthcare services and patients.

OP5

STIMULATING ADIPONECTIN REDUCES BONE EXPRESSION OF NERVE GROWTH FACTOR IN MYELOMA-BEARING MICE; A NOVEL APPROACH TO COMBAT BONE PAIN IN CANCER-BONE DISEASE

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Bone pain is a major clinical feature of tumour growth within the skeleton, and the most common presenting symptom of cancers such as multiple myeloma. Nerve growth factor (NGF) is a secreted protein, which promotes sprouting of nociceptive nerves, is commonly induced by skeletal disorders such as cancer metastases and osteoarthritis and positively correlates with pain sensitization. NGF can be targeted to prevent bone pain in prostate cancer and arthritis models and negatively correlates with adiponectin. Adiponectin is expressed by healthy adipocytes and bone marrow stromal cells (BMSCs), and we have shown that high adiponectin is protective against myeloma development. We hypothesize that adiponectin suppresses NGF expression within the bone environment, and pharmacological targeting of the adiponectin pathway may be used therapeutically to reduce tumour burden while also treating the associated bone pain.

We employed an in vitro and in vivo approach using bone cell lines (2T3 and ST2) and the well-established 5TGM1 mouse model of myeloma, treated with existing anti-tumour therapies (proteasome-inhibitor bortezomib or DNA-alkylating agent melphalan) or the peptide L-4F, which promotes endogenous adiponectin transcription and release. Using ELISA, we detected an increased level of NGF in untreated myeloma-bearing mice, and a significant 2-fold reduction in tumour-induced NGF following treatment with L-4F, but not bortezomib or melphalan, despite similar reductions in tumour burden from each treatment.

5TGM1 myeloma cells do not express NGF, but high levels of NGF were detected in osteoblasts and BMSCs in vitro, suggesting that the in vivo response to increased adiponectin may be dependent upon cellular interactions within the tumour-bone microenvironment. In support of this notion, osteoblast expression of NGF increased by 2.67 fold in response to co-culture with myeloma cells, whilst disruption of adiponectin signalling in osteoblasts using siRNA knockdown of both adiponectin receptors also increased NGF expression by 2.69 fold ($p<0.05$). Our study suggests that myeloma-induced NGF expression by osteoblasts is a likely cause of nerve dysregulation and bone pain. Adiponectin-based therapies may provide an improvement over traditional drugs by acting on the skeletal microenvironment, to reduce bone pain as well as reducing tumour burden.

OP6

PATELLOFEMORAL JOINT MORPHOLOGY IN NORMAL AND REPLACED KNEES

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Detailed knowledge of the shape and orientation of the trochlea groove is critical when considering potential causes of anterior knee pain, especially in the post-operative knee replacement patient. It is possible that abnormal movements of the patella might cause pain via abnormal loading of the surrounding soft tissues.

The aim of this study was to compare the path of the trochlear groove in both normal and replaced knees. The bony and cartilagenous trochleae from 3T MRI scans of 20 normal subjects were compared with patients with either a standard total knee replacement (TKR), an anatomical TKR or patellofemoral joint replacement (PFJR). Following segmentation using Mimics (v. 14.1, Materialise, Belgium) and re-alignment using Geomagic Studio (v. 11.0, Geomagic, USA), the path of the trochlea groove throughout flexion was measured using a custom routine in Matlab (R2010b, The MathWorks Inc., Natick, MA, USA).

In normal knees, in flexion between 0 degrees and 60 degrees, the mean path of the trochlear moved laterally for both cartilage and bone. Between 60 degrees and 90 degrees flexion the path was central for bone but moved medially for cartilage. These paths were not reproduced by any of the knee prostheses; the anatomical TKR and PFJR had a medially orientated trochlea, whilst the standard TKR showed a central (straight) path throughout flexion.

Non-parametric statistical tests (paired analysis), analysing trochlear paths during flexion, revealed that only the standard TKR was not statistically different from the native cartilage trochlear path (p equals 0.14 - 0.74). In all other combinations of bone versus prosthesis and cartilage versus prosthesis, the trochlea showed statistically different paths (p less than 0.05).

These data suggest that the shape of the articulating surfaces of the distal femur are not re-created following joint arthroplasty. Abnormalities in trochlear shape may lead to abnormal patella kinematics. It is likely that anterior knee pain following knee arthroplasty is secondary to an unfamiliar patella path dictated by the shape of the prosthesis.

OP7

THE ROLE OF MICRORNAS IN FUNCTIONAL OSTEOMIMICRY IN PROSTATE CANCER CELLS

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Prostate cancer (PCa) cells predominantly metastasize to bone and the complex crosstalk between PCa cells and osteoblasts (bone-forming cells) and osteoclasts (bone-destroying cells) results in an increase in tumour growth and worsening of bone disease. An understanding of the mechanisms by which PCa cells metastasize to bone can identify the aggressive fraction of PCa resulting in earlier intervention, and reveal new therapeutic opportunities. The ability of PCa cells to express bone cell-specific features, termed osteomimicry, could potentially explain the osteotropic nature of PCa bone metastases. The aim of this study was to determine the role of microRNAs in prostate cancer osteomimicry.

The bone metastatic PCa cell line ARCaPM, and its non-bone metastatic counterpart ARCaPE, were cultured in control or osteogenic medium. Mineralization and alkaline phosphatase (ALP) activity were detected by staining and a quantitative fluorescence assay respectively. Small RNA-sequencing was performed on Trizol-extracted total RNA at the Wellcome Trust Centre for Human Genetics, Oxford. mRNA expression was determined by real-time PCR.

ARCaPM cells deposit calcium phosphate, demonstrated by Alizarin Red S and Von Kossa stains; in contrast, ARCaPE cells do not mineralize. Expression and activity of the osteoblast marker ALP was detected in ARCaPM, but not ARCaPE, and ARCaPM cells also express other osteoblast markers including osteocalcin and osteopontin. Knockdown of SNAI1 in ARCaPM resulted in a significant ($p<0.05$) decrease in mineralization. Small RNA-seq revealed significant differences - 68 miRs were up-regulated and 57 down-regulated more than two-fold in ARCaPM over ARCaPE. Stable overexpression of miR-203 in ARCaPM resulted in significantly decreased expression of RUNX2 and ALP mRNA ($P<0.05$), ALP enzyme activity ($p<0.01$) and decreased mineralization.

Our data demonstrate that bone metastatic PCa cells exhibit a specific functional osteomimicry, resulting in PCa cell mineralization and suggesting that PCa cells may directly contribute to the osteosclerosis associated with such bone metastases. ARCaPE/M are a model for Epithelial to Mesenchymal Transition (EMT) in PCa progression and the differences in osteoblast-like features between these two cell lines indicate a role for EMT in osteomimicry. Further, the differential expression of microRNAs between ARCaPE and ARCaPM suggests an important role for miR-mediated regulation in PCa osteomimicry.

OP8

BIOMECHANICAL CHANGES FOLLOWING HIGH TIBIAL OSTEOTOMY

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High tibial osteotomy (HTO) surgery is performed as a treatment for medial compartment knee osteoarthritis (OA). The joint is realigned with the intention of relieving pain and shifting loads from the diseased medial to the lateral knee compartment. The aim of this study is to identify the efficacy of HTO surgery in modifying knee loading and determine any changes in the contralateral limb. Two clinically relevant markers of medial compartment loading and OA severity were investigated. These are the peak external knee adduction moment (EKAM) and knee adduction angular impulse (KAAI).

Bi-lateral knee function during gait was evaluated for 10 subjects pre-HTO surgery (height: 1.71±0.11m, mass: 85.9±15.9Kg, KL grade: 3-4). 6 subjects were re-assessed between 6 to 9 months following unilateral HTO using the opening wedge approach. Ethical approval was granted by the Research Ethics Committee for Wales and Cardiff and Vale University Health Board. 49 light retro-reflective markers were positioned in a modified Cleveland clinic marker set and 3D motion capture (Qualisys, Sweden and Bertec Corporation) used to record 6 trials of level gait at self-selected speeds. Biomechanical models of each subject were created in Visual3D (C-motion, Inc) to compute temporal, kinematic and kinetic data. Statistical analysis was performed using one-way ANOVA.

Following HTO, a statistically significant reduction in EKAM (Pre: 3.13 ± 1.21 %BW.h; Post: 1.55 ± 0.55 %BW.h) and KAAI (Pre: 1.17 ± 0.44 %BW.h.s; Post: 0.42 ± 0.27 %BW.h.s) was identified, indicating decreased load through the medial knee compartment. The mean percentage reduction in EKAM for the operative limb post-HTO surgery was 49.77% (maximum: 74.75%; minimum: 29.03%). There was a statistically significant improvement in both the Oxford Knee Score (20%) and Knee Outcome Survey (15%) indicating improved patient perceived function. A decrease in EKAM and KAAI for the contra-lateral non affected limb was identified, though these results were not statistically significant.

This pilot study has identified biomechanical changes following HTO and these factors are being investigated further with larger cohort numbers as part of an ongoing study.

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OP9

FUNCTIONAL SEGREGATION OF IMMUNOMODULATORY AND DIFFERENTIATION-COMPETENT MESENCHYMAL STROMAL CELL POPULATIONS

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Mesenchymal stem/stromal cells (MSCs) offer broad therapeutic options for bone and joint therapy, including immunomodulation and skeletogenic differentiation potential, however MSC heterogeneity and undefined functional MSC sub-classes has hindered progress. We adopted an immortalisation and cloning strategy to identify functional variance in human MSC subpopulations, initially isolating >100 clonal MSC lines and focusing on four (termed C101, C102, C201 and C202) with different behavioural traits. All lines were CD29, CD44, CD73, CD90, CD105 and CD166-positive, and CD34/CD45-negative, typical characteristics assigned to MSCs. C101 and C201 were capable of tri-lineage differentiation and C101 spontaneously differentiated towards osteogenesis with prolonged confluency. C102 and C202 showed no evidence of skeletogenic differentiation. Over 6,000 transcripts were differentially expressed between the four lines and parental MSCs ($P<0.05$, >2-fold) with clustering algorithms grouping C101 with C201, closest to primary MSCs, whilst C102 and C202 clustered independently. C101 and C201 were enriched in genes representative of mesodermal MSCs (PRRX1, PDGFRA, KITLG, GDF5) whereas C102/202 were enriched in angiohematopoietic genes (FLT1, KDR, TEK, EDN1, VWF, MCAM) indicative of alternative developmental origins. Significant differences in metagenes representing cell-ECM interactions, RTK signalling, adipogenesis and endochondral ossification were identified. Immunomodulatory genesets such as cytokine/inflammatory and interferon/TNF signalling were enriched in the non-differentiating (C102/202) lines and strikingly, 61% of genes altered upon exposure of MSCs to proinflammatory cytokines were also differentially expressed between C202 and the parental MSCs, suggesting an unstimulated immunomodulatory function in rare, poorly-differentiating MSC subpopulations. Using ELISAs we confirmed increased secretion of IL-7, a cytokine essential for lymphopoiesis, in C102/C202 versus C101/C201. Heterogeneous primary MSCs secreted low levels of IL-7, with negligible expression in dermal fibroblasts. To identify IL-7-positive MSC subpopulations in vivo, we used an IL-7Cre Rosa26-eYFP lineage-tracing mouse model. YFP-positive cells were found in bone marrow at perivascular and endosteal-lining locations with an approximate 4% frequency. 91% of femoral osteocytes, terminally-differentiated cells of the osteogenic lineage, were YFP-negative, indicating they derived from an IL7-negative MSC progenitor. These findings provide strong evidence for the existence of bone marrow subsets of differentiation-competent MSCs and resident, immunomodulatory MSCs, which will determine bone health, autoimmunity and selection criteria for orthopaedic therapy.

OP10

TECHNICAL VALIDATION OF A NOVEL FLEXIBLE CAPACITIVE FORCE SENSOR FOIL

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Force measurement within implants is important for validation of mechanic and kinematic models of human joints and for implant development. Up to now, capacitive sensors have not been applied within orthopaedic implants. They have the advantage that they are flexible, preventing implant wear, cheap, and they can be made very small in a standardised industrial process. This paper contains the results of the technical validation of a novel type of flexible capacitive force sensor; firstly to characterise the sensor and then to assess it in situ.

A capacitive force sensor with four electrodes was designed. It consisted of three copper layers, interspaced with Polyimide (PI). The thickness of the assembly was 0.1 mm. The sensors were manufactured using standard PI. In both tests, load was applied with a hydraulic load machine (Dartec DC10, Zwick, UK). Capacitance was measured with an AD7746 Capacitance to Digital Converter (Analog Devices, Norwood, MA, USA). Glass cubes with dimensions 6.5x6.5x6.5mm were glued on top of the electrodes, to apply a repeatable and uniform pressure to the electrodes only. Five such sensors were tested, and every test was repeated 5 times. The load had a static magnitude of 100N, plus a dynamic sine wave load of 33N peak. The sine waves had frequencies 0.1-1Hz in steps of 0.1Hz, and 1-10Hz in steps of 1Hz. Static loads were applied for 5 minutes and the the dynamic load was applied for 10 seconds per frequency. A sine function was fitted to the data. In the in situ experiment, the electrode foils were moulded within a large orthopaedic UKR bearing. Instrumented total knee gait and step patterns were obtained from orthoload.com (K1L11018_80p_gait, k2l_290409_1_109p), normalised to the load experienced by one condyle, and applied to the instrumented bearing.

The normalized amplitude and phase response were approximately flat from 0.1-1 Hz, and then a decayed linearly from 1-10Hz, with 0.02 * 1/Hz for magnitude and 4.5 degrees/Hz for phase response.

Embedded foil measurement results: preliminary results show >0.99 correlation for gait and >0.97 step up/down data, between applied load and measured capacitance change.

The measurements have shown that the novel capacitive load sensor provided accurate measurements of load within a physiological frequency range. Therefore, PI foil is a promising material for embedded orthopaedic sensors.

OP11

DOES PHYSICAL ACTIVITY INFLUENCE ADOLESCENTS BONE MINERAL DENSITY WITH EQUAL EFFECT AT ALL BMI LEVELS? FIT FUTURES 2010-2011, THE TROMSØ STUDY

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Introduction

Bone mineral density (BMD) is a strong predictor of future fracture risk and achievement of high peak bone mass is essential. Body mass index (BMI) and physical activity may both influence achievement of peak bone mass; the aim of this study was to explore the effect of physical

activity on BMD at different BMI levels in adolescents aged 15-18 years.
Methods
In 2010-2011 all first year comprehensive school students in the Tromsø region were invited to participate in the Fit Futures study, an expansion of the Tromsø study. Altogether 508 girls and 530 boys attended the survey providing an attendance rate > 90 %. BMD at the total hip and femoral neck was measured as g/cm2 by DXA (GE Lunar prodigy, Lunar Corporation, Madison, WI, USA). Lifestyle variables were collected by self-administered questionnaires and interviews; leisure time physical activity by the Gothenburg instrument. Multiple regression analyses were used to explore the association between physical activity and BMD according to BMI levels. The analyses included 471 girls and 498 boys and BMI was adjusted according to Coles cut off points for adolescents and children.
Results
The mean BMD was 1.059 (SD 0.123) g/cm2 at the total hip and 1.067 (SD 0.123) g/cm2 at the femoral neck in girls and in boys 1.113 (SD 0.147) and 1.100 (SD 0.150) g/cm2 at the total hip and femoral neck, respectively. Early sexual maturation, higher BMI and higher self-reported physical activity levels were positively associated with BMD at both femoral sites in girls. Variables significantly associated with bone mass in boys were similarly BMI, physical activity levels and timing of sexual maturation but also smoking and alcohol intake. In girls, physical activity levels were significantly associated with BMD in the underweight and normal weight classes (p<0,01), and not in the overweight and obese. In boys, physical activity levels were significantly associated with BMD in those who were normal weight and overweight for age (p<0,01), and not in the underweight or obese groups.
Conclusion
Physical activity seems to influence BMD differently at different BMI classes in adolescents, with different relationships seem in males and females.

OP12

ASSESSING SURGICAL OUTCOMES WITH INDUSTRY METRICS: A PROSPECTIVE COHORT STUDY OF 6912 ARTHROPLASTY PATIENTS

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Background
The Net Promoter Score, widely used in industry, has been introduced to the NHS as the 'friends and family test' - an overarching metric of patient satisfaction and to measure performance. The questionnaire asks 'customers' if they would recommend a service or products to others. Our aims were (1) to determine net promoter scores for joint arthroplasty, (2) compare with direct measures of patient satisfaction, and (3) evaluate which factors contribute to net promoter response.
Methods
6912 individuals undergoing primary lower limb joint replacement at a single centre over a five year period (Jan 2006 - Dec 2011) were prospectively assessed. Net promoter score, PROMS (Oxford Hip or Knee Score and SF-12 score), multi-faceted patient satisfaction questionnaire, demographic data and length of hospital stay were recorded. Multivariate regression was performed to determine which factors could predict an outcome of 'promoter' and 'detractor' at 1 year post-surgery. Significance was accepted at p = 0.1 to accommodate the confounding effect of other variables.
Results:
Net promoter scores for knee and hip replacements were 49 and 71 respectively. Strong correlation was seen between overall satisfaction and whether the patient would recommend the operation to another (r = 0.637), though regression of these factors was modest (R2 = 0.406). Only 4 factors were relevant to the net promoter response: pain relief (OR 2.13, CI 1.83 - 2.49), meeting expectations (OR 2.57, CI 2.24 - 2.97), hospital experience (OR 2.33, CI 2.03 - 2.68) and arthroplasty type (OR 2.31, CI 1.68 - 3.17). These factors drove a model able to explain 95% of the variation in net promoter score.
Conclusions:
This is the first analysis of net promoter scores for arthroplasty. THA and TKA demonstrate scores comparable with the most successful commercial organizations. The Department of Health describe their new metric as a measure of satisfaction. This is not completely accurate, as though although related, these concepts are not identical. Pain relief, meeting expectations, hospital experience and arthroplasty type are the only factors relevant in determining net promoter response. Factors thought to influence clinical outcome such as depression, comorbidities, age or gender carry no influence with this metric.

OP13

REDUCED EXERCISE BENEFITS IN BONE IN OLDER AGE AND IN EXERCISE BEGUN IN ADULTHOOD

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Upper limb muscle and bone strength decreases with age, whilst fracture rates increase - their incidence being similar to that in the lower limbs. Tennis playing is associated with large benefits in muscle and bone size and strength in the racquet arm - however little is known about whether these exercise benefits are affected by age. Therefore, peripheral quantitative computed tomography (pQCT) scans were taken at radius, ulna and humerus mid-shaft and distal radius in both arms of eighty-eight veteran tennis players (mean age 63.8±11.8y). Whilst muscle size and strength were negatively associated with age, there were no negative age effects on bone strength in compression, bending or torsion. Large side differences in muscle and bone size and strength in favour of the racquet arm were found - most pronounced were a 13±10% higher distal radius bone mass and 23±12% larger humerus cortical area (both $P < 0.001$). Side differences were less pronounced in older players, despite no age effect on training volume ($P = 0.195$) - most obviously in the humerus where bone mass and bending/torsional strength side differences were 43-47% less in 80- than 40-year olds ($P < 0.01$). Similarly, side difference in maximal hand grip force was 45% lower at the older age ($P < 0.001$) - suggesting reduced muscular force rather than mechanical sensitivity of bone may be responsible for the reduced osteogenic effect of exercise at older age. Players who began tennis playing in adulthood displayed smaller bone strength side differences (particularly 80-290% greater periosteal circumference and bone CSA - $P < 0.05$ at all sites). Side differences in hand grip force were 41% smaller in adult starters - supporting previous conjecture that maximal force is limited after epiphyseal closure in an attempt to prevent damage to soft tissues. In summary, regular tennis participation results in considerable benefits in bone and muscle size and strength in veteran players. However, these benefits are lower in older age, and when exercise is begun in adulthood i.e. after epiphyseal closure. These results reinforce the importance of regular, appropriate physical activity during childhood and adolescence.

OP14

MEASURING BONE MINERAL DENSITY OF THE PROXIMAL FEMUR FOLLOWING TOTAL HIP ARTHROPLASTY USING NOVEL, REGION-FREE DUAL ENERGY X-RAY ABSORPTIOMETRY ANALYSIS SOFTWARE (DXA-RFA)

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Dual energy X-ray absorptiometry (DXA) is considered the reference method to measure bone mineral density (BMD) following total hip arthroplasty (THA). Current analysis methods require the use of regions of interest (ROIs) to measure average BMD change for each region. These regions are produced based on assumptions of the pattern of bone loss around prostheses and do not necessarily reflect the areas where greatest change occurs. The aim of our work was to develop and validate a novel DXA analysis method that measures pixel-by-pixel

femoral periprosthetic BMD from a DXA scan, removing the need for ROIs.

DXA scans from 29 previously recruited subjects whom had undergone primary THA within the previous 13 months were studied. Subjects underwent two DXA scans of the proximal femur on the same day, separated by a period of repositioning. Identical copies of every scan were made and software precision was assessed for identical and repositioned scans. The repeatability was expressed as coefficient of variance (CV) using a 7 ROI model and the results compared to those obtained in the original study.

Analysis of identical scans gave a BMD CV between 0.3% and 0.8% for individual ROIs, with a net CV for the whole femur of 0.3%. Comparing repositioned pairs provided a net CV of 1.7% (range 2.9% to 3.7%) which was similar to that produced using traditional analysis methods, which obtained a CV of 1.6% (range 1.5% to 3.6%). Scans were then aligned to a master template, designed to allow average BMD at each pixel to be calculated across a sample of patients. The CV was 3.8% with a range of 2.0% to 7.1% when comparing scans before and after alignment.

The software showed acceptable precision for the measurement of periprosthetic BMD in the proximal femur on a pixel-by-pixel basis. Due to the softwares improved resolution compared to ROI models it can be used to identify the exact areas where greatest BMD change occurs following THA. It ensures no data is masked as a result of averaging and is flexible to different prosthesis designs, as different templates can be produced for different study samples.

OP15

CHARACTERISING MUSCULOSKELETAL PHENOTYPE IN A POPULATION WITH LOW FRACTURE INCIDENCE

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The relationship between muscle and bone may be an important determinant of fragility fracture risk. Low calcium intake, low BMD and high PTH are characteristic in The Gambia yet documented fragility fractures in older people are low. We aimed to characterise the relationship between grip strength and BMD, bone area and bone strength, and muscle area and density using peripheral QCT.

Males (M) and females (F) aged less than or equal to 40 years residing in rural areas of The Gambia were recruited stratified by 5 year age-band. Outcome measures were: 4% radius(R4)-total(tot) and trabecular(trab) BMD, total area(totA); 66% radius(R66)-bone mineral content (BMC), cortical(cort) BMD, totA, medullary area(medA), stress-strain index(SSI), muscle area(muA) and muscle density(muD). Grip strength (N/kg) was measured by hand dynamometry. Independent samples t-tests tested sex differences for anthropometry. Univariate regression tested the relationship between between grip strength and bone and muscle outcomes. ANCOVA tested sex differences after adjustment for age, height, weight, grip strength. Sex effects are presented as % mean difference [95% CI].

Four-hundred and eighty-eight individuals (239M) were recruited, mean (SD) age M 60.8(12.3)yrs, F 61.1(12.6)yrs, height M 1.7(0.1)m, F 1.6 (0.1)m; weight M 59.9(10.3)kg, F 54.7(10.3)kg. Grip strength was positively associated with all bone measures (M: R2 2-9%, P<0.001-0.03; F: R2 4-29%, p<0.001-0.006). Grip strength was associated with CSMA in M and F and only muD in F (muA R2 27%(M), 29%(F), p<0.001: muD 1%, NS, 5%, p<0.001).

M had higher tot(22%[17,28]) and trab(40%[31,48]) BMD and totA (5%[0.4, 9]) than F at R4. At R66 M had larger bones (13%[10,16]) with higher BMC (32%[27,38]), BMD (3%[2,5]) and SSI (32%[27,38]); medA was not different to F (-0.3%[-1,1]). Grip strength was a significant predictor in the model for BMC, TotA R4, R66 and SSI (bone strength).

These data provide evidence for a relationship between grip strength and bone in M and F. Greater bone strength in males is through larger bones, thicker cortices and greater BMD/C. Determining how these relationships change with age may help to understand why this population has low incidence of fracture.

OP16

CESSATION OF AMBULATION, NOT STEROID EXPOSURE RESULTS IN A DRAMATIC LOSS OF TRABECULAR BONE DENSITY IN BOYS WITH DUCHENNE MUSCULAR DYSTROPHY (DMD)

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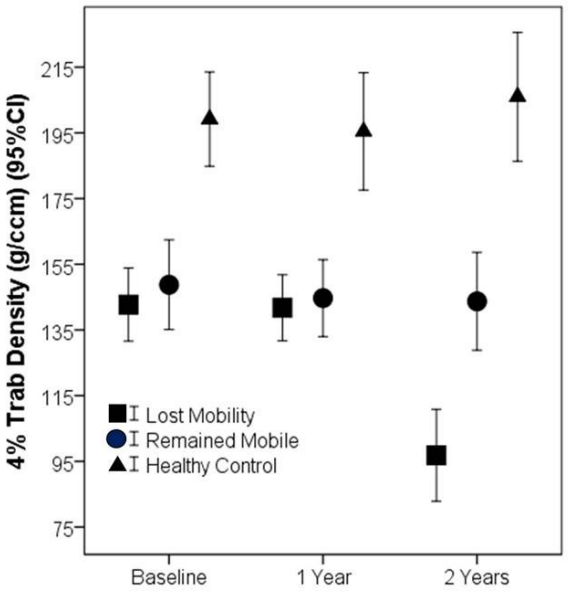
Steroids are currently used to improve muscle strength and prolong ambulation in boys with DMD although the effect on bone health is still unclear. The aim of this study was to compare bone strength in healthy children and boys with DMD and investigate the interaction between diminished muscle function, loss of ambulation and high dose oral steroids.

Fifty children were studied, 14 healthy boys (HB), 13 boys with DMD who remained ambulant (DMD-RA) and 23 boys with DMD who lost ambulation (DMD-LA). All boys with DMD were taking oral steroids. Peripheral quantitative computed tomography was used to measure bone geometry, density, strength and muscle mass of the non-dominant tibia. Measurements were made at baseline, 12 and 24 months at the distal metaphysis and mid diaphysis sites. Differences between the three groups were evaluated using ANOVA and a repeated measures model.

There were no significant differences in age between the groups, mean age was 9.4(2.7), 8.7(1.9), 8.8(1.8) years for HB, DMD-RA and DMD-LA, respectively. There was no significant difference in steroid exposure between DMD groups. However, boys who lost ambulation had significantly lower muscle function. Healthy boys had significantly greater trabecular bone density (26%) than boys with DMD (p<0.001). However, the rate of change of trabecular bone density was only significant for boys who lost ambulation. By 2 years non-ambulant boys had 51% less trabecular bone than their healthy age matched peers (See Figure).



Previous work has suggested that loss of ambulation can be predicted by assessment of muscle function. Since this study highlights the dramatic loss of bone density with loss of ambulation it is likely that functional assessment can help identify the point at which medical intervention to strengthen bones should be considered.

OP17

3D MENISCUS KINEMATICS THROUGHOUT KNEE FLEXION AND LOADING: A NOVEL IN VIVO MRI STUDY OF THE KNEE

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Meniscal tears are a recognised risk factor for osteoarthritis, often occurring during twisting or squatting [McDermott, 2006]. In-vivo understanding of meniscus biomechanics is limited to evaluating single 2D MR slices [Vedi, 1999], low resolution imaging [Shefelbine, 2006] and does not considering joint loading or transverse knee rotations. The purpose of this study is to i) assess the feasibility of high resolution 3D MR imaging of the knee during joint loading in a closed bore scanner, ii) quantify 3D in-vivo menisco-tibial kinematics during passive knee flexion, iii) during axial load and iv) transverse plane TFJ motion.
Novel, 3D, high resolution MR Imaging (Signa HD-xt 3.0T, GE Medical Systems, USA.) of 5 healthy knees was performed using a custom MR compatible loading device. The knee was positioned in extension and 25 degrees flexion under load and 0, 25, 50 and 130 degrees of passive flexion. Internal and external tibial rotation with load was additionally imaged. 3D positions each meniscus relative to the tibia were investigated by segmenting and creating 3D models of each structure from MR scan data (Simpleware Ltd, UK). Meniscal motion is described by the co-ordinate position of the meniscus centroid relative to a tibial local co-ordinate system created by identifying anatomical landmarks on 3D bone models reconstructed from additional 3D MR imaging of the ankle.

n=1	Medial Meniscus		Lateral Meniscus	
Knee flexion/ degrees	Posterior translation/ mm	Medial translation/ mm	Posterior translation/ mm	Medial translation / mm
21.2	3.2	-0.4	1.0	3.2
46.9	3.3	-1.3	4.5	3.6
142.1	10.6	-1.3	9.2	6.4

Table 1: Anterior-posterior and medio-lateral meniscus centroid translation for one healthy volunteer with changing passive knee flexion angle.

n=4	Medial Meniscus		Lateral Meniscus	
Knee flexion/ degrees (SD)	Posterior translation/ mm (SD)	Medial translation / mm (SD)	Posterior translation/ mm (SD)	Medial translation/ mm (SD)
2.7 (2.8)	0.44 (2.1)	-0.1 (2)	0.2 (1.4)	0.3 (3.2)
26.7 (4.5)	1.2 (2.3)	1.4 (2.9)	2.4 (2.8)	-2.6 (2.3)

Table 2: Anterior-posterior and medio-lateral meniscus centroid translation with axial loading for 4 healthy volunteers (relative to the unloaded neutral position meniscus centroid locations)

This study demonstrates the feasibility of imaging the knee under load from which 3D in-vivo menisco-tibial kinematics can be quantified. In contrary to majority of published findings, the medial and lateral menisci appear equally mobile. Loading introduces additional posterior motion of both menisci, up to 4.6mm (medial) and 5.2mm (lateral) in the flexed knee. Large variability (SD) is observed between subjects (> repeatability errors). Transverse knee rotations introduce additional meniscus centroid translations (up to 6.3mm).The high resolution imaging and 3D modelling methods of our study additionally suggest that the posterior horns of both menisci rotate towards the centre of the tibial plateau during posterior displacement of the meniscus centroid which has not been previously reported, likely a mechanism working in conjunction with the ACL to resist posterior motion of the femur relative to the tibia during knee flexion.

P 1

BASELINE BONE MINERAL DENSITY AND BONE RESORPTION MARKERS AMONGST PREOPERATIVE HIP AND KNEE ARTHROPLASTY PATIENTS

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Osteoporosis and increased bone resorption rates at the time of surgery may prove to be significant factors influencing the outcome of hip or knee arthroplasty surgery, predisposing to complications of aseptic loosening and periprosthetic fracture. This unique prospective study of 234 patients awaiting hip or knee arthroplasty, measured baseline bone mineral density using DEXA scans, and baseline bone resorption activity using urinary deoxypyridinoline (DPD).
The prevalence of hip osteoporosis, as defined by total hip T-score of -2.5 SD or less, amongst hip and knee arthroplasty patients, was found to be low (2.9%). The true figure may be up to 8.3% however, when allowing for those already on bisphosphonate therapy. This prevalence is still lower than that expected in the general population of a similar age.
Mean total hip T-score (-0.22, sd 1.31) was within normal limits, and bone mineral density amongst the study cohort was normally distributed. Standard deviations were large however, indicating a broad range of results.
Median urinary deoxpyridoline/creatinine was raised in males 7.0 (IQ Range 5.7-9.1), but normal in females 6.8 (IQ Range 5.2-9.2). 64% of the study population had raised DPD/creatinine ratios, whilst 7% had more than twice normal ratios. This suggests abnormal bone metabolism and raised resorption in a large proportion of arthroplasty patients.
Further work is now required to determine the significance of bone health on arthroplasty outcome, and whether BMD and/or DPD should be routinely measured prior to surgery. We have the ability to alter bone metabolism and mineral density through bisphosphonate therapy, and at present only a small proportion (18.8%) of DEXA determined osteoporotic patients are being treated prior to arthroplasty surgery.

P 2

CLASSIFICATION OF KNEE OSTEOARTHRITIS ACCORDING TO SPATIO-TEMPORAL GAIT ANALYSIS

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Background - Knee osteoarthritis (OA) is a common disease with estimated prevalence of 30% in patients over the age of 60. Classification systems have focused on radiology and clinical symptoms alone. The gait changes in patients suffering from knee OA are well documented in the literature and include, among other, lower step length and velocity. In this work we are suggesting a new classification method for knee OA based on spatio-temporal gait analysis.
Methods - Gait analysis of 2,900 patients from AposTherapy dataset (AposTherapy, Herzeliya, Israel) suffering from knee OA were included in the study. Men and women were analysed separately. The analysis included three stages - clustering, classification and clinical validation. Clustering of gait analysis data by the kmeans method created four groups. Two thirds of the patients were used to create a simplified classification tree algorithm. The model's accuracy was checked by using the remaining one third of the patients. Clinical validation of the classification method was done by SF-36 and WOMAC questionnaires.
Results - The clustering algorithm divided the data to four groups according to gait analysis severity. The classification tree algorithm used stride length and cadence as predicting variables for classification. The correct classification accuracy was 89.5%, and 90.8% for women and men, respectively. Clinical scores of the WOMAC and SF-36 questionnaires correlated well according to severity group. For example, in women, the rate of total knee replacement within a year after the gait analysis was 1.4%, 2.8%, 4.1% and 8.2% for knee OA grades 1-4, respectively.
Conclusion - Spatio-temporal gait analysis can be used to classify patients with knee OA according to disease severity. The most

differentiating variables for classification are stride length and cadence. Furthermore, gait analysis based on disease grading correlated with clinical data of pain, function and quality of life.

P 3

A NON-INVASIVE FOOT-WORN BIOMECHANICAL DEVICE AND TREATMENT FOR PATIENTS WITH HIP OSTEOARTHRITIS

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Purpose: Physical therapy and biomechanical interventions for patients with hip osteoarthritis (OA) should aim to reduce pain, improve function and restore or maintain gait patterns close to normal. The purpose of this study was to evaluate the effect of a biomechanical therapy on the pain, function, quality of life and spatio-temporal gait patterns of patients with hip OA.

Methods: 60 patients with hip OA were examined before and after twelve weeks of using the biomechanical device and treatment (AposTherapy). Patients were evaluated using the WOMAC questionnaire for pain and function and the SF-36 Health Survey for quality of life. Patients also underwent a computerised gait test.

Results: After twelve weeks of treatment, a significant improvement was found in the patients velocity, step length and cadence. (p 0.001).WOMAC-pain, WOMAC-stiffness and WOMAC-function subscales were significantly improved compared to baseline (P 0.001). SF-36 physical score subscale improved significantly (P=0.007), whereas the SF-36 mental subscale improved but did not reach the statistical significance threshold.

Conclusions: Patients with bilateral hip OA treated with the examined therapy for twelve weeks showed statistically and clinically significant improvements in pain, function and gait patterns. These findings suggest that this treatment may be an additional useful tool for conservatively treating patients with hip OA. Further RCT studies are needed to evaluate the effect of the examined therapy on hip OA population.

P 4

ENHANCED BIOMECHANICAL CLOSED KINETIC CHAIN THERAPY INTERVENTION IN THE REHABILITATION OF PATIENTS AFTER TOTAL HIP ARTHROPLASTY

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Introduction: Some patients after total Hip Arthroplasty (THA) may suffer from a limp and periarticular discomfort due to muscle weakness. Physiotherapy is important in restoring muscle strength. Evidence-based guidelines on rehabilitation after THA are scarce. We examined the immediate and longer term effect of closed kinetic chain exercises (AposTherapy) causing controlled perturbations over gait parameters after THA.

Materials and Methods: Thirty five patients were prospectively followed during the study. Gait parameters were measured at initial evaluation, after 15 minutes of therapy and after 3 months of treatment. SF-36 and WOMAC scores were filled by patients before treatment and after 3 months of treatment.

Results: Gait velocity, single limb support (SLS) and step length of the operated leg significantly improved after a single 15 minute treatment (72.9 cm/s vs. 87.6 cm/s, 33.3% vs. 35.2 % of gait cycle and 45.8 cm vs. 50.2 cm, respectively, p0.001) and furthermore after 3 months of treatment (72.9 cm/s vs. 108.5 cm/s, 33.3% vs. 38.2 % of gait cycle and 45.8 cm vs. 56.7 cm, respectively, p0.001).

Forty three percent of patients had a normal gait velocity after 3 months of treatment; SF-36 and WOMAC scores significantly improved after 3 months of treatment (p0.008).

A significant reduction was seen in the self-reported levels of pain, function and quality of life following 3 months of treatment.

Discussion: Using a closed kinetic chain exercise implemented by a foot-worn platform is useful for patients post THA. Improvement in gait, limb functionality, stiffness and pain may be seen after a single session and may be more noticeable after 3 months of treatment.

P 5

REHABILITATION MODIFYING INTERVENTION BY ENHANCED BIOMECHANICAL CLOSED KINEMATIC CHAIN THERAPY IN PATIENTS AFTER TOTAL KNEE ARTHROPLASTY

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Introduction: Many factors contribute to suboptimal results of Total Knee Arthroplasty (TKA). Little is known regarding the value of post surgical rehabilitation after TKA. We examined the effect of an enhanced closed kinematic chain exercises program on gait patterns and clinical outcomes among patients with a lack of progress in their post surgical rehabilitation.

Materials and Methods: Twenty two patients were prospectively followed during the study. Gait spatio-temporal parameters were measured at initial evaluation, after 15 minutes of therapy and at 3 months. WOMAC and SF-36 were filled by patients before treatment and after 3 months of treatment (AposTherapy).

Results: WOMAC and SF-36 scores significantly improved after 3 months of treatment. Gait velocity, single limb support (SLS) and step length of the operated leg significantly improved even after a single 15 minute treatment. Normal gait velocity appeared among 36% of patients after 3 months of treatment.

Conclusions: We found rehabilitation using enhanced closed kinematic chain biomechanical therapy to be potentially beneficial for patients post TKA who experience a suboptimal rehabilitation course. The expected time for a consolidated improvement is 3 months.

P 6

A RETROSPECTIVE SINGLE SURGEON REVIEW OF 338 PATIENTS WHO UNDERWENT METAL-ON-METAL HIP ARTHOPLASTY OR RESURFACING

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Background

Total Hip Replacement (THR) is a very successful, and commonly performed procedure that relieves pain and restores function in the arthritic hip joint. However, in younger patients, there is an increased risk of failure, due to excessive wear of the artificial joint. In order to reduce wear, new materials were introduced, and these included Metal-on-Metal (MoM) implants

Recently there has been some concern over MoM implants, particularly due to increasingly poor outcomes and also over metal ions found in the blood stream.

Aims

This retrospective service review aimed to study the relationship between levels of Cobalt-27 (Co) and Chromium-24 (Cr) in whole blood and patient outcome after MoM THR or resurfacing.

Methods

338 patients attended a follow up clinic at least 6 months after a MoM hip replacement or resurfacing. 52 patients with bilateral implants were excluded. At this appointment the patients had blood taken and were evaluated using the Oxford Hip Score (OHS). The blood was analysed and the levels of Co and Cr were recorded.

The results were analysed using Spearmans correlation and the Kruskall-Wallis one-way analysis of variance.

Results

The preliminary results show a very weak correlation between the Co and Cr levels and post-operative OHS. However there was a moderate correlation with differences between pre and post op OHS and Cr levels, but this was not seen with Co. There was a significant relationship between Co levels and the categories of OHS, (Excellent-Poor), a high Co was seen in those patients in the worse OHS categories, but this was not seen with Cr. .

Discussion

The results show that there is a relationship between patient outcome and Co and Cr levels after hip surgery using MoM bearings. This relationship is clinically relevant because it could lead to earlier recognition of a failing implant

P 7

LONG-TERM OUTCOME OF BISPHOSPHONATE THERAPY IN PATIENTS WITH MILD PRIMARY HYPERPARATHYROIDISM

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Background. Bisphosphonates therapy has been used in patients with primary hyperparathyroidism (PHPT) with the aim of increasing bone mineral density and reducing fragility fractures. However, there is still no conclusive evidence to guide practitioners in the appropriate medical management. We evaluated the effect of bisphosphonate treatment on serum adjusted calcium, bone mineral density, rate of fragility fracture in patients with PHPT and high risk of fragility fracture who were not suitable for parathyroidectomy.

Methods. All the patients with primary hyperparathyroidism not suitable for parathyroidectomy attending the bone metabolic clinic at the Royal Liverpool and Broadgreen University Hospital for bisphosphonate treatment were included. Data were collected from clinic letters and laboratory information system.

Results. 50 patients with PHPT were included. The mean age was 74 years, 94% females. Baseline mean serum adjusted calcium (2.74mmol/L), bone mineral density for lumbar spine (L2-L4, mean T-score -2.5), and left femoral neck (mean T-score -2.1). 14 patients (28%) had bone fragility fracture at baseline. After a mean 4.8 years of bisphosphonate treatment, there was a significant decrease in serum adjusted calcium to 2.60mmol/L ($p<0.001$), lumbar bone density increased significantly to -2.1 ($p=0.013$). There was no change in rate of bone fragility fracture ($p= 0.167$). Out of the 14 patients who had fractures at baseline, only one patient had a recurrent fracture. A total of 7 patients (including the recurrent fracture) had new fractures after mean duration of 6 years (standard deviation 2.5 years) of bisphosphonate treatment. 1 fragility fracture (rib) occurred after 11 years of treatment. Additionally, there was a significant decrease of urine calcium to creatinine ratio (0.70 and 0.55 before and after treatment, respectively, $p<0.0001$)

Conclusion. Bisphosphonate therapy significantly improves the Lumbar spine bone mineral density and prevents further increase in rate of fragility fracture. Additionally, the therapy significantly reduced serum adjusted calcium and urinary calcium excretion. However, the results also suggest that prolonged treatment with bisphosphonate may contribute to fragility fracture in PHPT. Further randomised controlled studies are needed to confirm the effect of bisphosphonate and vitamin D supplementation on fragility fractures and determine the optimum duration of bisphosphonate treatment in PHPT.

P 8

IS THE FRACTURE RISK ASSESSMENT TOOL (FRAX) SENSITIVE ENOUGH FOR TERIPARATIDE, AND WHAT IS THE OSTEOPOROTIC VERTEBRAL FRAGILITY FRACTURE RATE DURING TERIPARATIDE TREATMENT?

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Background:

Osteoporosis is a progressive systemic skeletal disorder, with low bone mass, and increased susceptibility to fracture, and is defined as a bone mineral density T-score of <2.5.

1 in 3 women have an osteoporotic fragility fracture in their lifetime, costing ?·1.73 billion annually.

FRAX estimates the 10-year major osteoporotic fragility fracture probability, to screen patients at risk of an osteoporotic fragility fracture and recommend suitable treatment. NICE recommends treatment protocol is based upon T-score, age and clinical risk factors for fracture, and not FRAX results.

NICE recommends teriparatide as a 4th line treatment for secondary prevention of osteoporosis in postmenopausal women. Teriparatide is an anabolic treatment, composed of the first 34 amino acid sequence of parathyroid hormone.

Aims:

Investigate FRAX sensitivity pre- and post-teriparatide treatment, and calculate a vertebral fracture rate for patients receiving teriparatide treatment at the University Hospital Llandough (DGH).

Method:

39 patients were used to investigate FRAX sensitivity pre and post-teriparatide treatment, and 15 patients were chosen to investigate vertebral fracture rate during teriparatide treatment.

Data was collected retrospectively from a database of patients who have received teriparatide treatment, and dual-energy X-ray absorptiometry scans. Data was input into a spreadsheet to calculate FRAX risks pre- and post-teriparatide treatment, and vertebral fracture rate during teriparatide treatment.

Results:

This case series found a vertebral fracture rate of 6.7%, directly comparable to the landmark study by Neer et al1 at 5.0%.

Post-teriparatide treatment the average neck of femur T-score decreased by 0.015. With 10 year risk of a major osteoporotic fragility fracture decreased on average by 0.97%, and 10 year risk of an osteoporotic fragility hip fracture decreased on average by 1.07% post-teriparatide treatment.

Conclusion:

The vertebral fracture rate while receiving teriparatide treatment for The University Hospital Llandough is 6.7% and correlates with the recommended studies1.

Results infer teriparatide treatment has minimal effect on neck of femur bone mineral density: FRAX is not sensitive enough to calculate an osteoporotic fragility fracture risk reduction post teriparatide treatment.

References:

1. Neer Et Al. Effect of Parathyroid hormone (1-34) on fractures and bone mineral density. The New England Journal of Medicine 2001;344:1434-1441.

P 9

PREVENTION OF INFECTIOUS COMPLICATIONS IN CHILDREN WITH BONE TUMORS AFTER THE ARTHROPLASTY

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Background: The treatment of bone tumors in children requires numerous courses of chemotherapy - both before and after surgery. An initial problem to be solved is providing venous access: comfortable for the patient and entailing minimal risk of infections. This is particularly important to prevent infection of bone implants in the joints. The best option is fully implantable venous port systems.

Materials and Methods: From 2008 to 2012 we observed 175 children with bone tumors of extremities (aged 3 years to 17 years). Sparing surgery (limb arthroplasty) was performed in 167 patients (95.4%): in 2008 - 24 patients, in 2009 - 34, in 2010 - 28, in 2011 - 44, in 2012 - 37. The lowest age of the patient, who underwent surgery for knee replacement - 3.5 years, the shoulder joint - 4 years. We have used venous ports since 2010 and implanted them in 80 (45.2%) patients with limb bone sarcomas: in 2010 - 5 (17.8%) patients, in 2011 - 39 (88.6%), in 2012 - 36 (97.2%). Subclavian catheters were implanted in 96 (54.8%) patients.

Results: Infectious complications developed in 18 patients with limb endoprosthesis (10.8%). There were 3 infected implants (12.5%) in 2008, 5 (14.7%) - in 2009, 3 (10.7%) - in 2010, 4 (9.0%) - in 2011, 3 (8.1%) - in 2012. Two-step re-arthoplasty was performed in 11 (61.1%) patients, conservative treatment (antibiotic therapy with Maxipime, Amikacin, Zyvox or Cubicin) helped to keep the implants in 7 patients (38.8%). In this early - developed within 3 months after the operation - infectious complications occurred in 64.3% of patients, delayed - from 3 months up to 2 years - 24.1%, and late - over two years - in 11.6%. Catheter-related bloodstream infection developed in 28 (29.1%) patients with subclavian catheters, while in patients with implantable venous ports such infections were not noted. The most common cause of catheter-related infections - S. epidermidis (71,8%) and S. aureus (18,2%), also inoculated when infected implants.
Conclusion: The introduction of implantable venous port-systems for the treatment of child patients with bone tumors has significantly reduced (1.8 times) the number of infectious complications and infections of limb prostheses, improving quality of life.

P 10

PREVENTION OF CATHETER-RELATED INFECTIONS IN CHILDREN WITH TUMORS OF THE MUSCULOSKELETAL SYSTEM

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Background: The treatment of musculoskeletal tumors in children requires numerous courses of chemotherapy that necessitate adequate vascular access. Implantable venous port-systems are free from many of the disadvantages associated with the use of external central venous catheters. Our goal was to reduce the occurrence of infectious and thrombotic complications in children with central venous systems.
Materials and Methods: From 2008 to 2012 we observed 281 patients with tumors of the musculoskeletal system aged 6 months to 17 years, for 147 (52.3%) of which implanted venous port systems were used and for 134 (47.6%) with external subclavian catheters. Estimated criteria: the development of catheter-related bloodstream infections and cases of catheter thrombosis. In cases of thrombosis, we injected the system with a 25,000 IU dose of urokinase with an exposure of 15 minutes. To seal the catheter between the usages, we used heparin or a solution containing taurolidin (no catheter-related infections were noted).
Results: Periportal tissue infection was observed in 3 cases (2.0%) of the patients with implanted venous ports, while the children with subclavian catheters puncture site infection was noted in 89 cases (66.4%). No catheter-related bloodstream infections were noted at children with venous ports. Thrombosis of venous ports was observed in 7 cases (4.7%), which caused by incorrect exploitation. The development of catheter-related bloodstream infections was noted in 18 cases (13.4%) at children with subclavian catheters. Subclavian catheter thrombosis was observed in 47 cases (35.0%). The treatment of complications caused in exploitation of a subclavian catheter required its replacement in 29 cases (21.6%), with the necessity of another general anesthesia. All venous ports worked satisfactorily. All cases of thrombosis were successfully treated.
Conclusion: The use of taurolidin solution to close the venous system in the intervals between treatments prevents infection. The treatment of catheter-related infections is more effective with a combination of taurolidin and urokinase, which provides lysis of the thrombus as a source of bacteria. The local use of a gel containing taurolidin at endoprosthesis infecting is possible. The number of complications is significantly higher in patients with subclavian catheters, which rises the risk of limb endoprosthesis infection.

P 11

MESENCHYMAL/STROMAL CELL DERIVATION BY NANOTOPOGRAPHICAL DIFFERENTIATION OF HUMAN STEM CELLS

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An unlimited source of bone-forming osteogenic cells would overcome a number of challenges faced by the fields of bone research and regenerative medicine. In culture, human embryonic stem cells (hESCs) exhibit rapid proliferation and a capacity for self-renewal with retention of pluripotency. Using a nanotopographical surface we have directed the mesenchymal differentiation of hESCs toward cell-types similar to skeletal stem cells offering an alternative source to human or animal bone marrow-derived cells.
Nanotopographical surfaces displaying 120nm diameter nanopits in a near square arrangement (300nm centre-centre spacing with 50nm displacement) were fabricated by injection moulding of polycarbonate. hESCs incubated on planar polycarbonate or near square polycarbonate show a loss of self-renewal marker expression (Nanog, OCT4, SOX2, TRA-1-60 and SSEA4). On near square nanotopography in the absence of chemical cues, differentiated cells exhibited enhanced expression of mesenchymal (BMP4), stromal (STRO-1, CD44, CD63) and early osteogenic progenitor (Type I Collagen, RUNX2 and Osteonectin) markers at levels above those detected in cells on planar surfaces.
Developmental events during nanotopography-directed differentiation were also explored. A reciprocal switch in E-cadherin to N-cadherin expression was observed during nanotopographical differentiation, a hallmark of epithelial to mesenchymal transition. DNA methylation within the OCT4 promoter region was enhanced in cells differentiated on planar or near square nanotopographies and upon differentiation by withdrawal of self-renewal media.
Nanotopographical-directed differentiation provides a unique, facile and innovative approach to mesenchymal differentiation of hESCs. The addition of chemical supplements to the media or prior induction of differentiation by embryoid body formation is not required, making this technique suitable for the production of cells for bone biology research, small molecule screening and regenerative medicine.

P 12

MATERNAL VITAMIN D STATUS IN PREGNANCY AND OFFSPRING BONE HEALTH: A SYSTEMATIC REVIEW AND META-ANALYSIS

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We performed a systematic review to elucidate whether 1) low maternal circulating 25(OH)-vitamin D [25(OH)D] during pregnancy is associated with adverse maternal and neonatal bone health; and 2) maternal supplementation with vitamin D in pregnancy leads to an improvement in these outcomes. Other disease outcomes were also assessed.
Major electronic databases were searched from inception till June 2012, together with hand-searching of bibliographies and direct author contact. Primary outcomes included: Maternal osteomalacia; Neonatal hypocalcaemia, rickets and reduced bone mass; secondary outcomes included: Maternal quality of life; Neonatal body composition and bone mass, later offspring health outcomes (including asthma, diabetes, immune disease). We performed systematic review and where possible combined study results using meta-analysis to estimate the combined effect size. All assessments were performed by two reviewers and according to UK CRD guidance.
16,841 citations were identified. After initial screening, 172 remained for detailed assessment, which resulted in 73 studies finally included in the review (10 clinical trials of maternal vitamin D supplementation). There was considerable heterogeneity between the studies and for most outcomes there was conflicting evidence; however modest positive relationships were found between maternal serum 25(OH)D and 1) offspring birth weight in meta-analysis of 3 observational studies using

log-transformed 25(OH)D concentrations after adjustment for potential confounding factors; 2) offspring cord blood or postnatal calcium concentrations in a meta-analysis of 6 intervention studies (all found to be at high risk of bias); and 3) offspring bone mass in 5 observational studies found to be of good quality, but which did not permit meta-analysis.
Although there was weak evidence to support a relationship between maternal 25(OH)-vitamin D status and offspring birth weight, bone mass and serum calcium concentrations, these findings were limited by their observational nature or low quality and risk of bias. High-quality intervention studies to investigate these outcomes would be appropriate, but the current evidence base is insufficient to directly inform clinical practice.

P 13

USING ASPIRIN FOR CHEMICAL THROMBOPROPHYLAXIS IN KNEE ARTHROPLASTIES, A RETROSPECTIVE STUDY

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The nice guidance for elective total knee replacements states that patients should be given mechanical thrombo-prophylaxis, and if no contraindications chemical thrombo-prophylaxis in the form of Dabigatran etexilate, Rivaroxiban, UFH, LMWH,or Fondaparinux sodium (CG92, 1.5.14, January 2010). In Practice administering oral agents has been the dominant practice as it reduces the nursing needs, and shortens hospital stay and is generally received better by patients. However there are well documented associated bleeding risks, and their effects are difficult to reverse in case of major bleeding. Our experience with oral factor 10 inhibitors used for thrombo-prophylaxis was marked with several patients developing complications necessitating return to theatre for wound washouts. This has led us to try a different protocol for thrombo-prophylaxis that we applied on our patients undergoing total and unicondylar knee replacements.
We applied mechanical thrombo-prophylaxis in the form of intermittent pneumatic pressure devices, and chemical thrombo-prophylaxis in the form of a dose of prophylactic LMWH pre-op, then 150 mg of Aspirin to start 24 hours after the surgery and to continue for 6 weeks, alongside GI cover with PPIs or antihistamines. We also administered local anaesthetics intra-operatively in line with the ERAS protocol thus encouraging early mobilization.
We have identified a cohort of 133 patients who underwent one of the aforementioned procedures in the same trust, and by the same surgeon, where this protocol was applied and examined their medical notes retrospectively with a mean follow-up period of 14 months, to identify the rate and percentage of patients who had thrombo-embolic events in the post-operative period. The rate was 2 of the 133 thus yielding a percentage of 1.5%.

P 15

NEUROPHYSIOLOGICAL FINDINGS OF LONG TERM COMPUTER USERS WITH MUSCULOSKELETAL PAIN

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Intensive computer work can increase the risk of developing musculoskeletal symptoms and disorders in the upper extremities. We have proposed that computer users have developed minor neural injury as a consequence of restricted gliding or compression of the nerves in the wrist region. The aim of this study is to compare sensory nerve conduction velocities for median, radial and ulnar nerves in the distal arms of symptomatic computer users with age, gender and height matched controls who do not use computer regularly.
The sensory responses were obtained by antidromically stimulating at the wrist and recording from the index finger (for the median nerve) and little finger (for the ulnar nerve) using ring electrodes. The sensory responses of the radial nerve were obtained by antidromically stimulating at the middle of the forearm adjacent to the cephalic vein and recording with a disk electrode that was placed between the first and second fingers.Questionnaires regarding work patterns and musculoskeletal symptoms were completed by the participants.Simple biometric measurements were also performed.
The sensory conduction velocities of the median and ulnar nerves at the dominant extremity were significantly delayed in the computer users (53.76±4.98 m/s and 52.66±6.53 m/s, respectively) compared to controls (64.96±4.12 m/s and 65.22±5.42 m/s, respectively). However, there was no statistical difference in conduction velocity of the radial sensory nerve between the control group and computer users (P= 0.375). The sensory conduction velocities of the median and ulnar nerves at the nondominant extremity were significantly delayed in the computer users (57.30±4.81 m/s and 49.47±5.54 m/s, respectively) compared to controls (63.01±7.27 m/s and 62.53±7.41 m/s, respectively). However, there was no statistical difference in conduction velocity of the radial sensory nerve between the control group and computer users (P= 0.396).
Our study shows that long term computer users have a tendency to experience median and ulnar sensory nerve damage. Sustained wrist extension and ulnar deviation may have resulted in stretching of these nerves across the wrist during computer mouse use and typing.

P 16

NOVEL NEURONAL CHANGES IN ROTATOR CUFF TENDINOPATHY (RCT)

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Introduction
Shoulder pain is the third most frequent cause of chronic musculoskeletal pain in the community and is usually caused by rotator cuff tendinopathy (RCT). The central and peripheral nervous system play an important role in both tissue homoeostasis and tendon healing. The Glutaminergic system is of key importance in driving the peripheral and central neuronal changes which increase the body's sensitivity to pain. No study to date has investigated the role of the glutaminergic system in human RCT.
We hypothesised that the peripheral neuronal phenotype would be altered in RCT, and would vary according to disease stage as measured by size of tear. The term peripheral neuronal phenotype is used to refer to refer to specific characteristics of the peripheral nervous system, neuronal mediators and the receptors for these mediators in peripheral tissue
Methods
Rotator cuff tendon specimens were obtained from 64 patients undergoing the surgical repair of rotator cuff tears. Control supraspinatus tendon was obtained from 10 patients undergoing surgery for anterior instability using an ultrasound guided biopsy technique. Patients with rotator cuff tears were divided into 2 groups: the small/medium group (3cm size) and the large/massive group (>3cm size). The tendon tissue was histologically stained using Haematoxylin and Eosin, and immunohistochemically stained with primary antibodies visualised using 3,3-diaminobenzidine (DAB). Image analysis was performed blindly by 2 observers using Image-J to quantify the amount of DAB positive staining. Data was non-parametric in distribution and Mann-Whitney U tests were carried out using SPSS with significance levels set at a minimum of $p<0.025$.
Results
There were significant changes in the peripheral neuronal phenotype in RCT. The Glutaminergic system was significantly up-regulated with an increase in Glutamate and changes in several related receptors in disease versus control ($p<0.01$). The standard deviation in nuclei count and mean cell nuclear area were both increased in disease ($p<0.01$) compared to controls. Tendon vascularity and cell proliferation were reduced in disease vs control ($p<0.01$). There were no significant correlations between pain scores and the peripheral tissue markers.
Discussion/Conclusion
The peripheral neuronal phenotype is significantly altered in rotator cuff tendinopathy (RCT) with clear changes in the Glutaminergic system in disease. These findings are novel and improve our understanding of pain and tissue healing in RCT, and provide novel therapeutic targets.

P 17

THE EFFECT OF SOFT TISSUE FAILURE AND TOTAL KNEE REPLACEMENT CONSTRAINT ON THE ENVELOPE OF LAXITY

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Objectives: Our aim was to quantify and display in a 3D graph, the envelope of motion at 0-110 degrees of knee flexion, how it changes as its soft tissue envelope fails and to what extent a standard cruciate retaining (CR) implant or posterior stabilizing (PS) implant restores and maintains this envelope.

Methods: 6 fresh frozen cadaver legs were mounted on a purpose built rig with the quadriceps, hamstrings and ITB loaded. Optical trackers were screwed onto the fixed femur and the mobile tibia. Motion was tracked using computer navigation. Envelope of motion was quantified under varus/ valgus, anterior drawer and internal/external rotation from 0 -110 degrees flexion. Displacements were measured sequentially in the native knee, after arthrotomy, ACL sectioning, insertion of CR implant, PCL then popliteus sectioning, insertion of PS implant, MCL and LCL sectioning. Effects were quantified using mixed effect statistical modelling.

Results: Significant effects of specimen, ligament sectioning and angle of knee at test were observed, more markedly in varus/ valgus and internal/ external rotation. In all cases there was an increase in laxity with increasing angle or ligament sectioning. Operator and movement cycle had no effect. Insertion of the CR implant increased stability within the joint, especially in internal/external rotation and anterior draw movement tests. Once the PCL was cut the CR implant only maintained stability in internal/ external rotation, demonstrating soft tissue envelope failure. Implantation of the PS implant restored stability, but failed as further soft tissue cuts were made. Minimal changes to the flexion arc occurred with soft tissue deficiency increase, but changed markedly upon implant insertion compared with the native knee.

Conclusions: Our results are similar to those of previous cadaveric studies but highlight the mid-flexion and high flexion behaviour of the knee. The limits to which a CR implant or PS implant can maintain knee stability are also quantified. With refinement, this model could potentially be used in vivo, as an interface to guide surgeons in addressing the soft tissues appropriately and selecting the correct implant.

P 18

FUNCTIONAL OUTCOME FOLLOWING WEBER B FRACTURES OF THE ANKLE

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Introduction: Ankle fracture outcome is not well understood, although the injury is common. There is a need for research on predictors of outcome and recovery patterns.

Methods: We conducted a retrospective study of ankle fractures in the Nottingham area. Patients were assessed at different time points following their surgery using the AOFAS, the Olerud and Molander and the VAS-FA functional outcome scores. In addition to these, qualitative data was collected during a structured interview.

Over the last 4 years, 1085 patients were operatively treated with ankle fractures. We selected isolated unimalleolar Weber B fractures. Patients presenting with comorbidities or under drug treatment were excluded to limit confounders. Analysis of outcome scores and subjective questions (45 patients) and outcome scores alone (6 patients) was undertaken.

Results: Mean age was 54.9 (SD ±18.3), M:F ratio was 2:3. The mean age of women was 58.9 (SD ±2.7, Men mean age was 48.7 (SD ± 4.8)), which is keeping with the literature. Mean outcome scores (maximum score=100) were AOFAS 79.2 (SD ±19), Molander 75.7 (SD ±25.6), VAS-FA 80.5 (SD ±19.3). The proportion of high scores peaked at 24-30 months postoperatively. Overall, 30 patients considering themselves fully recovered, whereas 15 did not. Most patients considered they reached a full recovery after 30 to 36 months postoperative. A significant difference in perceived outcome (p-value=0.0091) was found between patients who exercised and those who did not. Patients with higher expectations for their recovery were found to have better outcome and lower pain scores: the AOFAS and Molander scores showed a 36% decrease in these pain scores whereas the VAS-FA showed a 22% decrease.

Conclusions: Ankle fractures, even when patients were closely matched, showed large variation in outcomes. Higher recovery expectations and exercise improve the chances of a successful recovery. Most patients (74%) reported a full recovery 24-36 months postoperative.

P 19

BONE MARROW STROMAL CELLS OF FEMALE BAG-1 HETEROZYGOUS MICE EXHIBIT REDUCED OSTEOGENIC POTENTIAL

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BAG-1 (Bcl-2-associated athanogene-1) is a multifunctional protein which, by its ability to bind multiple partners, regulates gene transcription and molecular signalling crucial for cell proliferation, differentiation and apoptosis. Expression of Bag-1 mRNA has been identified in several organs, with cartilaginous tissues showing highest expression in the developing mouse embryo. Furthermore, in long bones of postnatal mice, expression of BAG-1 is detected in both chondrocytes and osteoblast-lineage cells. Bag-1 null mice are embryonic lethal, while mice heterozygous for Bag-1 (Bag-1+/-) are viable.

The study aims to elucidate the function of BAG-1 in osteoblast development by examining differences in osteogenic differentiation of bone marrow stromal cells (BMSCs) from Bag-1+/- and wild-type mice.

BMSCs isolated from femora and tibiae of 14-week-old Bag-1+/- and wild-type mice were cultured for 28 days in basal and osteogenic (100ng/ml rhBMP-2) media. Cells were harvested for analysis of proliferation by DNA assay, apoptosis by TUNEL staining, expression of differentiation stage-specific osteogenic genes by qPCR, Alkaline phosphatase (ALP) specific activity and Osteocalcin (OCN) production by ELISA.

BMSCs from Bag-1+/- female mice failed to undergo robust osteogenic differentiation in response to BMP-2, unlike BMSCs from wild-type female mice that responded to BMP-2 by significantly upregulating ALP and OCN expression in day 28 cultures. Interestingly, in osteogenic cultures, BMSCs from Bag-1+/- female mice proliferated at a significantly higher rate throughout 28 days of culture in comparison to their wild-type counterparts. In contrast, BMSCs from male Bag-1+/- mice exhibited robust osteogenic differentiation, which was comparable to the osteogenic response by BMSCs from male wild-type mice. In osteogenic cultures, BMSCs of Bag-1+/- and wild-type male mice proliferated significantly between days 1 and 14 of culture, while cell proliferation decreased significantly between days 14 and 28 of culture. In both female and male mice, no statistically significant differences in cell apoptosis were observed between the different groups of BMSC cultures.

Thus, in female mice heterozygous for Bag-1, proliferation of BMSCs was enhanced at the expense of osteogenic differentiation. These studies indicate an important role for BAG-1 in osteoblast development and the need to understand the role of interacting factors modulating gender differences.

P 20

SITAGLIPTIN ATTENUATES OVARIECTOMY INDUCED OSTEOPOROSIS IN RATS

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Background and purpose

Osteoporosis is one of the most prevalent metabolic bone disorders especially among postmenauposal women. Diabetes mellitus may be associated with development osteoporosis. Hence, the purpose of the current study was to investigate the effect of Sitagliptin (dipeptidyl peptidase inhibitor IV) on ovariectomy induced osteoporosis in rats.

Design and method

The study was conducted on 40 female rats that were divided into four groups each composed of 10 rats: Group 1 sham operated group, group 2 ovarectomized (OVX) group, while group 3 and group 4 were OVX rats treated with estrogen replacement therapy (ERT) and sitagliptin respectively for eight weeks. Blood samples were collected at the end of eight weeks for measurement of serum alkaline phosphate (ALP), calcium, phosphorus, osteocalcin and malondialdehyde (MDA). Urine samples were collected for measurement of urinary deoxypyridoline (DPY)/creatinine.

Results

The OVX-rats showed a significant decrease in serum calcium, a significant increase in serum ALP, osteocalcin, MDA and urinary DPY)/creatinine levels when compared to the sham operated group. Such biochemical alterations induced by ovariectomy were significantly ameliorated by administration of EHT and sitagliptin respectively.

Conclusions

Sitagliptin was found to be effective in decreasing bone resorption, increasing bone formation and hence reducing ovariectomy induced osteoporotic changes. Thus, the use of sitagliptin, as anti-diabetic agent, in postmenauposal women should be encouraged.

P 21

THE EFFECT OF VITAMIN D STATUS AND PTH ON THE RESPONSE TO ZOLEDRONATE

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Studies suggest that optimum vitamin D status is required for the maximal effect of bisphosphonates on the skeleton. Calcium and vitamin D supplements are routinely prescribed with bisphosphonates. We investigated the relationship between vitamin D status, serum parathyroid hormone (PTH) concentrations with the change in bone mineral density (BMD) following intra-venous Zoledronate at 1 year. We carried out a retrospective analysis of 111 patients mean age 70[13] years, 89F, 12M who had been prescribed Zoledronate for osteoporosis. We measured BMD at the lumbar spine (LS) and total hip (TH), serum 25 (OH)vitamin D, PTH, bone turnover markers (plasma CTX, P1NP) at 1 year, prior to the second infusion. The clinical indications for iv Zoledronate were intolerance/contra-indications (49.5%) and poor response to oral bisphosphonate (50.5%). Serum 25 (OH)vitamin D was mean[SD] : 70.8 [26.9] nmol/L, PTH: 44.8[18.1] ng/L, CTX: 0.156[0.104] ug/L, P1NP: 25.8[18.2] ug/L. Percentage change in BMD was mean [SEM] 1.05 % [0.5] at the TH and 2.6% [0.5] at the LS. 25 (OH)vitamin D was < 50 nmol/L in 21.6% of the study population. A significant correlation was observed between PTH with 25 (OH)vitamin D ($r=-0.334$, $p=0.001$) and CTX ($r= 0.315$, $p< 0.05$). There was a significant relationship between CTX and P1NP ($r= 0.540$, $p<0.001$). No significant correlation was observed between 25 (OH)vitamin D and % change in BMD. In contrast, there was a significant correlation between % change in BMD at the TH with PTH ($r= -0.25$, $p=0.02$) and LS with CTX ($r= -0.223$, $p<0.05$). Patients with PTH concentration <44ng/L had significantly higher increases in TH BMD compared to those with PTH > 44 ng/L (1.9 [0.83] v/s -0.43 [0.81], $p =0.04$). In summary, our data suggest that PTH influences the effect of Zoledronate on bone resorption and BMD. Therefore maintaining vitamin D status for optimisation of PTH concentrations is important to maximise the anti-resorptive effect of Zoledronate and possibly improve BMD outcome.

P 22

THE RELATIONSHIP BETWEEN INTACT PTH AND BIOINTACT PTH (1-84) WITH BONE AND MINERAL METABOLISM IN PRE-DIALYSIS CHRONIC KIDNEY DISEASE (CKD)

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Abnormalities in PTH are implicated in the pathogenesis of bone abnormalities in chronic kidney disease (CKD) -mineral bone disorder (CKD-MBD). PTH concentrations are important in clinical decision and management. This emphasises the importance of providing an assay which measures biologically active PTH. We compared concentrations of intact PTH with biointact PTH (1-84) in CKD and end stage renal disease (ESRD) and investigated the relationship between the 2 PTH assays with bone and mineral laboratory parameters and bone mineral density (BMD) in CKD. We assessed 140 patients (61 in ESRD and 79 with CKD stage 1-4) in this cross-sectional study. We measured biointact PTH (1-84) as well as routine biochemical parameters on all subjects. In the CKD cohort, bone turnover markers; bone alkaline phosphatase (BAP) and tartrate resistant acid phosphatase (TRACP)-5b and bone mineral mineral density (BMD) were also determined. In ESRD, intact PTH concentration was significantly higher compared to biointact PTH (1-84) (422 [443] v/s 266 [251] pg/mL, ($p < 0.001$) with an average bias of 60%. In CKD, intact PTH concentration was also higher compared to biointact PTH (1-84) (79[55] v/s 68[49] pg/mL $p < 0.001$) with an average bias of 18%. Only the biointact PTH (1-84) assay showed any significant correlation with serum calcium concentrations ($r = - 0.26$, $p < 0.05$) and phosphate ($r=0.25$, $p<0.05$) in CKD. Following multilinear regression analysis and adjustment for all significant co-variables, only eGFR, BAP and 25 (OH)vitamin remained significantly associated with intact PTH and biointact PTH (1-84). The strength of association was stronger between BAP and biointact PTH (1-84) (biointact PTH (1-84) : $p=0.007$, intact PTH : $p=0.01$). In adjusted analyses, only biointact PTH (1-84) was significantly associated with BMD at the FARM ($p=0.049$). The study confirms the differences between intact PTH and biointact PTH (1-84) in ESRD. Whilst there may be similarities in the diagnostic ability of both intact and biointact PTH (1-84), our data suggest that biointact PTH (1-84) assay may better reflect bone metabolism and BMD in CKD. Further longitudinal studies are needed.

P 23

THE EFFECT OF A LOADING DOSE (300,000 IU) OF VITAMIN D2 (ERGOCALCIFEROL) ON CIRCULATING PRO-RESORPTIVE INFLAMMATORY CYTOKINES IN VITAMIN D INSUFFICIENCY

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Close interactions exist between the immune system and the regulation of skeletal remodelling through a network of inflammatory cytokines. The effects of Vitamin D on the skeleton may be mediated, in part, by its modulation of these factors. We hypothesized that the acute increases in bone resorption following loading doses of vitamin D may involve vitamin D mediated up-regulation of these inflammatory pro-resorbing cytokines. The objective was to investigate changes in plasma concentrations of cytokines known to promote osteoclastogenesis including tumour necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), IL-6, IL-17, IL-8, granulocyte macrophage colony stimulating factor (GM-CSF), the chemokine monocyte chemoattractant protein-1 (MCP-1) following a bolus dose (300,000 IU) of vitamin D2 in subjects with vitamin D insufficiency. Circulating concentrations of the cytokines, 25 (OH)vitamin D, 1,25 (OH)2 vitamin D, the Wnt inhibitor; DKK1and bone turnover markers were measured in 39 subjects . 1,25 (OH)2vitamin D increased markedly following supplementation. We found significant correlations between 25 (OH) vitamin D ($r=0.4$ $p=0.016$) and 1,25 (OH)2vitamin D ($r=0.39$ $p =0.02$) with plasma CTX (marker of bone resorption). TNF-(alpha) and IL-1(beta) increased significantly after treatment at 3 months ($p<0.05$). Significant correlation was seen between IL-17 ($r= 0.41$, $p =0.017$) and

IL-6 (r =0.31, p=0.07) with serum phosphate at 3 months. The results suggest that up-regulation of pro-resorptive cytokines following loading doses of vitamin D, when supra-physiological concentrations of active vitamin D are attained , may lead to increases in bone resorption.

P 24

AUDIT OF DENOSUMAB FOR THE PREVENTION OF OSTEOPOROTICS FRACTURES IN POSTMENOPAUSAL WOMEN; ADHERENCE TO THE NICE TA204 GUIDELINES AND IMPROVEMENTS IN T-SCORES.

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Osteoporotic fragility fractures are prevalent amongst post-menopausal women. The human monoclonal antibody, Denusomab, can be used for primary and secondary prevention of such fractures, as an alternative to oral bisphosphonates. It is important to identify patients suitable for treatment with Denosumab. This can be achieved with the NICE TA204 guidelines.

We aimed to assess the compliance of Denosumab treatment for primary and secondary fracture prevention in outpatients treated at New Cross Hospital, Wolverhampton, with the NICE TA204 guidelines. Additionally, the use of DEXA scans and improvement in T-scores pre and post Denosumab treatment was assessed.

We identified all patients who received Denosumab treatment between March 2011 - January 2013. For each patient we assessed compliance with the NICE TA204 guidelines using the audit guidance pro-forma. DEXA scan T-scores were also assessed pre and post- treatment with Denusomab.

We identified 9 and 80 patients receiving Denosumab for primary and secondary prevention, respectively. All patients were female, age 53-94.

Criteria for age were not met in the primary prevention category, with 3 patients under the specified age of 65. Additionally, 1 patient did not meet the specified criteria for T-score. 3 primary prevention patients received a DEXA scan pre and post- treatment; all had an improved lumbar and hip T score. 100% compliance was achieved for all criteria for secondary prevention. 12 of these patients received a DEXA scan pre and post- treatment; of these 7 and 10 experienced an improved T-score at the hip and lumbar vertebrae, respectively. Average improvement in T-score was 0.13 g/cm2 at the hip and 0.74cm2 at the lumbar vertebrae.

Overall, full compliance with the NICE TA204 guidelines for secondary prevention was achieved. Compliance with the primary prevention guidelines was lower due to a lack of alternative available treatments. Denosumab showed a good response in improving T-scores in primary and secondary patients. However, not all patients underwent a DEXA scan pre and post treatment because not all had completed a year of treatment at the time of study. Recommendations include adhering more closely to the guidance for primary prevention regarding age, T-score and independent clinical risk factors.

P 25

LOCALISATION AND IDENTIFICATION OF BACTERIA IN THE SKIN OF PATIENTS AFTER SURGICAL SKIN PREPARATION

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Background

The bacteria usually causing surgical site infection in arthroplasty are also common skin flora, and evidence indicates that they usually gain access to the prosthesis during surgery. The patients' skin remains the primary source of infection, with Staphylococcus aureus, S. epidermidis and Propionibacterium acnes most frequently implicated. Effective skin preparation has the potential to reduce post-operative infection, although there is a lack of consensus regarding which antiseptic is best. Recent evidence suggests the superiority of alcoholic chlorhexidine, although studies have focused on sampling the skin surface despite evidence that sub-epidermal bacterial populations remain following antisepsis. This study aimed to determine the efficacy of a unitized alcoholic chlorhexidine preparation in reducing surface and sub-epidermal bacteria from the skin of patients undergoing orthopaedic surgery.

Materials and Methods

Following Ethics approval and informed consent, a pilot study recruiting patients undergoing hip and knee arthroplasty was undertaken. The surgical site was prepared using a unitized applicator containing 2% chlorhexidine gluconate in 70% isopropyl alcohol (Chloraprep). After preparation and before incision, swabs for aerobic and anaerobic culture and biopsies for culture and sectioning were obtained. A validated neutralising solution was used. Bacteria were identified from positive cultures using microbiological techniques and microscopy.

Results

Sixteen patients consented to participate and swabs and biopsies were obtained from primary knee (n = 10) and primary hip (n = 6) arthroplasties. Bacteria grew on culture of 25% of swabs (staphylococci, propionibacteria, corynebacteria and streptococci), and 56% of full thickness skin biopsies (Micrococcus and Bacillus spp, P acnes, S epidermidis and Staphylococcus warneri). Viable Gram-positive bacteria were identified microscopically in all skin sections.

Conclusions

Findings suggest that pre-operative skin preparation using alcoholic chlorhexidine does not sterilise patients' skin with viable bacteria remaining beneath the surface that have the potential to cause surgical site infection. Surgeons need to be aware of this and to adapt their surgical technique to avoid coming into contact with the patients' skin, including the cut edges, when performing joint arthroplasty . Further study is now required to compare alcoholic chlorhexidine with alcoholic povidone-iodine (PVP-I).

P 26

THE INFLUENCE OF SUGGESTION ON PAIN SENSITIVITY MEASURED USING QUANTITATIVE SENSORY TESTING (QST)

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Pain is a common disabling symptom with discriminative and affective mechanisms that are modulated through both pharmacological and psychological means. The affective component allows pain to be described as a subjective experience influenced by emotions, memories, personality and cognitive functions that can be altered through the use of suggestion to induce a placebo/nocebo effect. We aimed to quantify the suggestion effect by measuring a change in pain threshold.

20 male and 20 female healthy volunteers were randomised to two gender equal groups with no significant difference in age or general health ($p>0.05$). Groups received written and verbal suggestion that they would experience either an increase (Group 1) or decrease (Group 2) in sensitivity to pain following application of the medicated gel to the knee. Assessments of changes in pressure pain thresholds (PPTs) were made using quantitative sensory testing (QST) before and after the treatment by the application of a quantified increase in pressure such that a painful stimulus threshold was defined through the participant pressing a trigger. This used a CE marked algometer (Somedic, Sweden). Measurements were made in triplicate at the knee test site 15 minutes post application of the medicated gel and at the proximal tibia and sternum as controls.

Baseline PPTs differed significantly depending on gender and anatomical site, with males and the knee having the greatest PPTs ($p=0.0174$, and $p=<0.0001$ respectively), corroborating previous findings. In females, comparison between Groups 1 and 2 showed a significant difference at the knee with a median decrease in PPTs of -39.6kPa in Group 1 compared with -7.1kPa in Group 2 ($p=0.0431$). All other changes in PPTs between pre and post treatment measurement did not differ significantly between Group 1 and 2 in either females or males ($p>0.05$). A degree of sensitivity was shown to develop at the knee, causing decreasing PPTs on repeat testing ($p=0.0159$).

This study is a useful pilot for clinical research investigating the effect of treatment explanations on changes in pain perception. The power of suggestion was evident in the negative written and verbal suggestion group eliciting a nocebo effect in females.

P 27

PATIENT REPORTED OUTCOMES FOLLOWING ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION: A COMPARATIVE STUDY OF CIVILIAN AND MILITARY PATIENTS.

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Introduction:

Anterior cruciate ligament (ACL) rupture remains a common injury among young, physically active patients. Military personnel have been awarded special attention in the ACL literature, with focus given to fast-track surgery and return to active military duty as surrogate for satisfactory functional outcomes. There is however paucity in studies comparing military and civilian patients reported outcomes following ACL reconstruction.

Methods:

Retrospective observational analysis conducted at the University Hospital Birmingham a major trauma centre which is also part of the Royal Centre for Defence Medicine. Data was recorded in the early to mid-term follow-up period. Knee-specific patient-reported outcomes were collected pre and post-operatively, using the Knee injury and Osteoarthritis Outcome Score (KOOS).

Results:

Clinical and functional outcomes of 45 patients over a period of 2 years were reviewed. A total of 39 patients were included in the analyses, six patients were lost to follow-up and had incomplete scores. Of the final cohort 67% (n=26) were civilian patients. The mean age was 27.4 years (range=17-47). Parameters of injury mechanism, severity, type of repair, and rehabilitation were similar in both groups. The minimal perceptible clinical improvement (MPCI) for the KOOS is 10 points. Better clinically significant improvement was seen among civilian patients for pain scores, 92% vs. 85%. In contrast military patients had better clinically significant improvement in knee specific symptoms 100% vs. 89%, and sport/recreation 92% vs. 77%. Analysis of variance revealed no statistically significant difference between military and civilian patients in relation to pre and post-operative KOOS scores adjusted for age and gender. There were no statistically significant correlations between post-operative KOOS subscale scores, interval to surgery or length of follow-up.

Conclusion:

In comparable pre-operative knee-specific patient-reported scores there was no difference in post-operative outcomes between military and civilian patient groups. This study supports the argument that surgical decision making in ACL reconstruction should remain tailored to individual patient, injury and demands put on knee function.

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MEDICAL STUDENTS CAREER INTENTIONS AND ATTITUDES TOWARDS LEARNING TRAUMA AND ORTHOPAEDICS

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Introduction:

The undergraduate medical curriculum in the UK offers on average two and a half weeks in dedicated musculoskeletal placements. All the while bone and joint problems account for up to 25% of a general practitioners work load. This study aims to identify various opportunities which students perceive as most conductive to their learning; and whether students interested in this speciality have a different attitude towards learning trauma and orthopaedic surgery.

Methods:

A survey instrument was distributed to consecutive students from the same university and following the same curriculum. This was designed to capture student perception of key learning environment and how they best acquired core knowledge. It also included questions on career motivation towards trauma and orthopaedic surgery.

Results:

Among the 157 respondents 35 (22.3%) expressed their interest towards a career in trauma and orthopaedic surgery. Fourth year medical students reported educational value for trauma and orthopaedic surgery revealed that bedside teaching with a consultant was perceived extremely useful by 57.8% (n=89). A similar ranking was awarded to small group teaching seminars and bedside teaching with a junior doctor or trainee by 54.5% (n=85) and 51.6% (n=79) of students respectively. In contrast, morning trauma meetings and operating theatre learning environments were perceived to be of low educational value. Seeing patients within the clinical setting and the quality of teaching received were reported as the most motivating factors in career interest towards trauma and orthopaedic surgery, rated 43.9% (n=69) and 35% (n=55) respectively. Students interested in trauma and orthopaedic speciality as a career choice ranked seeing patients, quality of teaching received, assisting surgery and subject matter as the most significant motivating factors.

Conclusion:

Perceptions of educational benefit derived from each learning environment vary among undergraduate medical students. Overall the most valuable learning environment perceived by fourth year undergraduate medical students is formal patient based teaching, and despite diverging speciality choices students showed similar learning needs. Targeted teaching planning and suitable learning environments are clearly needed, in an era of abundant economic pressure certain clinical areas which were traditionally considered beneficial to undergraduate students need to be reassessed.

P 29

THE IMPACT OF ACHIEVING MAJOR TRAUMA CENTRE STATUS ON OPERATIVE TIME AND THEATRE UTILISATION: EXPERIENCE FROM A UK TRAUMA CENTRE.

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Introduction:

The reconfiguration of major trauma services is anticipated to provide clear benefits in clinical outcomes among the severely injured patients. All those concerned with service planning and provision are therefore inevitably watching the impact such reconfiguration will have on workload and resources. The aim of this study was to determine the effect of major trauma status in our centre on theatre time and operating department utilisation as well as the impact on routine orthopaedic trauma workload.

Methods:

The study was performed at the university hospital Birmingham a dedicated major trauma receiving centre in the west midlands. The study period followed the recent UK reconfiguration of regional trauma networks and included the first consecutive three months of the reconfiguration. We analysed data collected from the patients records, operating theatres database and the regional trauma audit research network.

Results:

A total of 276 cases were reviewed with a mean age of 44 years range (17 to 93), 76 % were male patients. The mean new injury severity score (NISS) was 27.5. Overall mortality rate was 5% at first 24 hours and 11% at 30 days. Median length of hospital stay was 13 days IQR (7-22) and median intensive care unit stay was 1 day IQR (0-6). There were 263 operative procedures carried out on 152 patients (55%). The mean number of operations per patient was two. 21% of operations were carried out as emergency during out-of-hours service. Theatre time utilisation in hours for the study period amounted to 264.24 out-of-hours and 773.48 normal-working hours. 45% (n=119) of operations were trauma and orthopaedic surgical procedures, and 60% of these were carried out on scheduled trauma lists. The latter equates to an average of 15.9 hours per week or the equivalent of four half-day theatre sessions per week. There was no signification correlation between the distribution of operating theatre time utilisation, and speciality, demographic or clinical parameters.

Conclusion:

Orthopaedic trauma surgery is the single largest speciality affected by the reconfiguration of regional trauma networks in the UK. Modern

trauma service has a much greater impact on scheduled compared to emergency theatre workload.

P 30

LYMPHOID AGGREGATES THAT RESEMBLE TERTIARY LYMPHOID ORGANS DEFINE A SPECIFIC PATHOLOGICAL SUBSET IN METAL-ON-METAL HIP REPLACEMENTS

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Introduction

Aseptic lymphocyte-dominated vasculitis-associated lesion (ALVAL) has been used to describe the histopathological lesion associated with metal-on-metal (M-M) hip bearings. We show for the first time that the lymphoid aggregates associated with ALVAL lesions harbour typical cellular and molecular features of tertiary lymphoid organs (TLOs), which are associated with several autoimmune and chronic inflammatory conditions.

Patients and Methods

Histopathological changes were examined in the periprosthetic tissue of 62 M-M hips requiring revision for adverse reactions to metal debris. Particular emphasis was paid to the characteristics and pattern of the lymphocytic infiltrate. Immunofluorescence and immunohistochemistry were used to study the classical features of TLOs in cases where large organised lymphoid follicles were present. Synchrotron X-ray fluorescence measurements were undertaken to detect localisation of implant derived Co and Cr ions/particles within the samples.

Results

Histopathological analysis of periprosthetic tissues from 62 M-M hip bearings was undertaken. Macrophages with variable amounts of intracellular metal wear debris were observed in 55 cases (89%). Macrophages in the remaining 7 cases (11%) contained no apparent particles. Lymphocytes were present in 51 cases (82%) with 11 samples (18%) lacking any significant lymphocytic infiltrate. Based on the type of lymphocytic infiltrates, three different categories were recognised; diffuse aggregates (n=26; 51%), T-cell aggregates (n=10; 20%) and organised lymphoid aggregates (n=15; 29%). Further investigation of tissues with organised lymphoid aggregates showed that these tissues recapitulate many of the features of TLOs with T-cells and B-cells organised into discrete areas, the presence of follicular dendritic cells, acquisition of high endothelial venule like phenotype by blood vessels, expression of lymphoid chemokines and the presence of plasma cells. Co-localisation of implant-derived metals with lymphoid aggregates was observed.

Conclusions

These findings suggest that in addition to the well described general foreign body reaction mediated by macrophages and a T-cell mediated type IV hypersensitivity response, an under-recognised immunological reaction to metal wear debris involving B-cells and the formation of tertiary lymphoid organs occurs in a distinct subset of patients with M-M implants. The significance of B-cells and TLO formation in M-M implant pathology and its correlation with disease severity and outcomes requires further evaluation.

P 31

OSTEOGENESIS IMPERFECTA IN AN ADULT METABOLIC BONE DISEASES CLINIC

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Background: Osteogenesis Imperfecta (OI) is an inherited disorder of type 1 collagen leading to skeletal fragility. Currently there are no guidelines on assessment and treatment in adults with OI.

Aim: To review clinical characteristics, biochemistry and treatment in adults with OI.

Methods: Clinical, demographic and laboratory data were obtained from IT systems and Bone Mineral Density (BMD) from the dual energy X-ray absorptiometry (DXA) database.

Results: 64 patients were identified (37 female (58%)). Mean age: females 38 (SE=2.9) years; males 32 (SE=2.7), p=0.17. 59 (92%) were characterised as type I OI with one type III and four type IV. 40% patients had been treated with bisphosphonates.

Most patients had a family history of fractures and multiple fractures with reducing frequency in adulthood.

DXA BMD (mean; SE) was higher in females (0.83g/cm2; 0.03) than males (0.71 g/cm2; 0.04, p=0.03); this was reflected in T (-2.0; 0.24 and -3.1; 0.4, p=0.01); and Z scores (-1.8; 0.26 and -3.03; 0.33, p=0.005) for females and males respectively. Similarly, the total hip BMD was 0.781g/cm2; 0.02 in females and 0.767; 0.04 in males (p=0.38).

Baseline P1NP was invariably suppressed. Vitamin D insufficiency was noted in 95% and deficiency in 5% of patients.

Conclusions: Many of the characteristics of adults with OI are similar to those previously described, but the apparently more severe disease in men is novel. The high prevalence of vitamin D insufficiency has implications for treatment and the low bone turnover raises concerns about the use of antiresorptive drugs in this population.

P 32

24,25-DIHYDROXYVITAMIN D3 IS A BONE FIDE VITAMIN D RECEPTOR AGONIST SUPPORTING HUMAN OSTEOBLAST MATURATION

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Background: Vitamin D receptor (VDR) agonists, which support human osteoblast (hOB) differentiation in the absence of bone resorption, are attractive agents in a bone regenerative setting. One potential candidate fulfilling these roles is 24,25-dihydroxy vitamin D3 (24,25D). Over forty years ago it was reported that 24,25D could stimulate intestinal calcium uptake and aid bone repair without causing bone resorption. VDR agonists co-operate with certain growth factors to enhance hOB differentiation and we discovered that lysophosphatidic acid (LPA) acted synergistically with 1,25-dihydroxyvitamin D3 to promote/secure hOB maturation. Whether 24,25D might act similarly in promoting cellular maturation has not previously been described. Methods: MG63 hOBs were co-treated with 24,25D (100nM) and a phosphatase-resistant (LPA) analog; 1-fluoro-3-hydroxy-4-(oleoyloxy)butyl-1-phosphonate (FHBP, 250nM). An assessment of cell number was performed using a combination of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium, innersalt (MTS, Promega, UK) and the electron-coupling reagent phenazine methosulphate (PMS). Evidence of maturation was assessed via osteocalcin (OC) expression (ELISA) and total alkaline phosphatise (ALP) activity. Cells were also processed to assess expression of the 1-alpha-hydroxylase (CYP27B1) by ELISA. To ascertain whether 24R,25D might bind to the VDR a competitive binding assay was employed in which a rat recombinant vitamin D receptor ligand-binding domain (amino acids 115-423) was incubated with increasing concentrations (1nM-1uM) of 24R,25D followed by treatment with [3H]-1,25D. Results: In isolation 24,25D inhibited proliferation and stimulated OC production. When co-administered with FHBP there were synergistic increases in ALP. A lack of CYP27B1 expression would suggest that 24,25D could serve as a bone fide VDR agonist without the need for 1-alpha-hydroxylation. The use of all-trans retinoic acid (ATRA) and the VDR antagonist (ZK159222) confirmed involvement of the VDR in the responses observed. Evidence from the VDR binding study confirmed that 24,25D bound to the VDR, but with

far less affinity than 1,25D. Conclusions: These are novel and encouraging findings which may help realise the future application of 24,25D in promoting osseous repair in an orthopaedic setting.

P 33

VITAMIN D STATUS IS POSITIVELY CORRELATED WITH DISTAL RADIAL TRABECULAR VOLUMETRIC BONE MINERAL DENSITY IN UK DWELLING POSTMENOPAUSAL SOUTH ASIAN WOMEN

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Previous research has found an association between vitamin D status (25-hydroxyvitamin D, 25(OH)D) and volumetric Bone Mineral Density (vBMD) in Caucasians[1-2]. However, there has been little research assessing this relationship in South Asians. Therefore, the aim of this work was to assess whether serum 25(OH)D is associated with bone geometry in postmenopausal South Asian and Caucasian women.

In summer 2010, 18 South Asian and 48 Caucasian women (aged 58 to 75 years) had pQCT scans (Stratec X2000L) undertaken of the radius (4% and 66% sites) and tibia (4%, 14% and 38% sites). Fasting blood samples were obtained for assessment of serum 25(OH)D.

Partial correlations assessed the relationship between 25(OH)D and bone geometry, adjusting for body mass index (BMI). At the 4% (distal) radius site, in Caucasians, there was a positive correlation between 25(OH)D status and bone mineral content (BMC) (r=0.404 p=0.008), total area (r=0.327 p=0.035), and trabecular area (r=0.327 p=0.034). For Asians, there was a significant positive relationship between 25(OH)D concentration and trabecular vBMD (r=0.547 p=0.035; see figure 1 for unadjusted data).

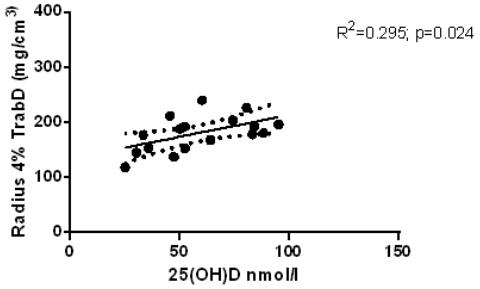

Figure 1: 25(OH)D concentration and radial (4%) trabecular density in Asians (n=18)- unadjusted data

In Asians there was no significant correlation between vitamin D status and any tibial bone parameter (p>0.05). However, at the 38% site in Caucasians, there were significant correlations between 25(OH)D concentration and bone mass (r=0.304 p=0.050). There were also significant positive associations between 25(OH)D and cortical area at the 14% site (r=0.353 p=0.022) and between 25(OH)D and trabecular area at the 4% site (r=0.336 p=0.029).

Overall, in Caucasians vitamin D status appears to be positively correlated with radial and tibial bone mass and size. In South Asians, vitamin D status appears to be positively correlated with distal radial trabecular density. Vitamin D was not correlated with tibial bone parameters in the Asians, but was associated with some tibial mass and area parameters in Caucasians. Further analysis is underway to assess possible explanations for these varying relationships between vitamin D status and bone geometry by ethnicity and bone site.

1 Pedone C, Napoli N, Pozzilli P, Lauretani F, et al (2010) Bone 46:1063-7

2 Sayers A, Fraser W, Lawlor A & Tobias J. (2012) Osteoporos Int 23:2117-2128

P 34

THE ELLIPSOID FACTOR

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There is currently no robust, meaningful measurement of trabecular bone plate/rod configuration which treats the trabecular phase as a mechanical continuum, which is resistant to artefacts induced by surface mesh creation or other parameters (e.g. bone volume fraction, BV/TV), and for which statistical comparison is meaningful. The structure model index (SMI), the de facto standard until now, fails when the surface mesh is non-representative, when BV/TV varies, and because SMI values relate non-linearly to shape. Concave surfaces have a negative contribution to SMI and are common in natural bone tissue, but are not accounted for in SMI's mathematical model. Shape descriptors based on discretizing the trabecular continuum into elements fail to measure junction regions appropriately and ignore the fact that individual trabeculae are never loaded in isolation from the continuum to which they belong. Here, I introduce the Ellipsoid Factor (EF) as a simply-defined, model-independent parameter which summarizes the 3-dimensional shape of trabecular bone and similar continua using maximal inscribed ellipsoids. The EF at a point in the structure is defined as the axis ratios of the greatest volume ellipsoid which fits within the structure and which contains the point. Ellipsoids that fit within rod-like structures are javelin-shaped (~prolate), while those fitting within plates are discus-shaped (~oblate). Ellipsoids at junctions tend to be more ball-shaped (~spherical). The composition of a structure can be displayed on a Flinn diagram, which shows the major:intermediate axis ratio on the y-axis and the intermediate:minor axis ratio on the x-axis. A rod-heavy structure will tend to concentrate to the upper left of the plot, while a plate-heavy structure will concentrate to the lower right of the plot. The structure can then be summarized in terms of the tendency towards prolate, oblate or spherical ellipsoids. Statistical analysis can be performed on binned or gated data or directly on Flinn diagrams from different treatment groups. EF is implemented as a menu command in the BoneJ plugin for ImageJ and is open source. The current implementation is functional: more efficient algorithms will likely surface in the future given the non-trivial nature of optimizing ellipsoid fitting.

P 35

OB ACTIVITY AND OC RESORPTION ABILITY AFFECTED BY GELATIN COMPOSITION

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New bone biomaterials are constantly being developed and improved to replace the current gold standard, an autograft. This is due to difficulty obtaining the patients own bone, and limited availability of cadaver bone. In order to produce biomaterials with similar properties to natural bone, natural materials such as gelatin composites are often used with promising results. While the use of gelatin in biomaterials is widespread, the effect of different gelatin extraction methods and gel strength (bloom) on the bone cell activity is not fully known.

Gelatin coatings were prepared using 5% solutions of Gelatin type-A (bloom #300), Gelatin type-A (bloom #90-110), Gelatin type-B (bloom #225) as well as 50:50 mixtures. Uncoated coverglasses and bovine serum albumin coatings were used as controls. MC3T3-E1 an osteoblast (OB) cell line and calvaria derived mouse OBs are cultured on coatings. Osteoclasts (OC) obtained from mouse bone marrow precursors were also generated on coatings in a co-culture system. The effect of the gelatin types on osteoblast differentiation and bone formation was measured via alkaline phosphatase (ALP) activity and Alizarin Red-S staining of mineralized matrix. OC differentiation was determined by the number of TRAP+ multinucleated cells, and OC activity by the number of OC with actin rings.

OBs were able to adhere to all gelatin substrates tested. All substrates supported OB attachment and subsequent mineralisation. All coated and uncoated surfaces supported ALP activity and the level varied both with extraction method and gel strength. However not all substrates supported OC differentiation, and the ability to resorb the surface was significantly affected by gel strength, as well as the gel extraction method used. There was a higher number of differentiated OC ($p<0.001$) present on the type-A high bloom gelatin (1627 OC/cm^2) compared to the type-A low bloom (338 OC/cm^2) and this was

reduced with a 50:50 mixture (24 OC/cm^2). Type-B gelatin did not support OC differentiation or actin ring formation.
OC activity is significantly affected by the gelatin type in the substrate. The effect of gelatin bloom and extraction type should be considered when developing biomaterials for use in bone replacement applications.

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GENE EXPRESSION SIGNATURES OF INDIVIDUAL SKELETAL STEM CELLS

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A variety of stem cells (SC) are present in the adult human bone marrow, including hematopoietic (HSC) & skeletal stem cells (SSC). Both HSC and SSC form a unique niche, which is defined by their mutual interaction. While for HSC complex gene expression patterns serve to isolate distinct progenitor states, a conclusive marker that identifies a bona fide SSC population within the bone marrow remains elusive. Nevertheless, surface markers such as STRO-1 enrich human bone marrow cells with skeletal differentiation potential. The aim of this work was to obtain single cell gene expression data for human SSC-enriched cell fractions in order to help decipher an expression signature for SSC and enhance their purification.
We applied a three step single cell fluorescence-activated cell sorting (FACS) strategy to sort individual antibody-labelled STRO-1 negative and positive cells from human bone marrow using a refined double sort strategy to enrich for highly expressing STRO-1 cells. These cells were then analysed with a microfluidic quantitative RT-PCR system (Fluidigm 96.96 Dynamic Array) to obtain expression data for 96 genes from each of a total of 288 individually sorted cells. The selection of genes, which encompassed HSC, SSC, pluripotent stem cell markers and other genes, grounded on the rationale to compare gene expression patterns throughout various stem cell populations and to reveal a distinct SSC identity.
We have generated a unique data set with 27648 gene expression values of individual cells within enriched and depleted SSC populations. We were able to distinguish STRO-1 expressing cells from non-expressing cells based on a multidimensional gene expression classifier that reveals the gene expression signature underlying skeletal stem cell identity. Ultimately, this signature can be exploited to isolate distinct SSC from the bone marrow. These refined SSC can be used in tissue engineering and cell based therapeutic approaches, where this pure and potent alternative can substitute for the heterogeneous cell populations currently employed.

P 37

THE HUMAN TISSUE EFFECTS OF COMMON ROTATOR CUFF TREATMENTS: IS STEROID DOING HARM?

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Introduction
Shoulder pain is the third most frequent cause of chronic musculoskeletal pain in the community and is usually caused by rotator cuff tendinopathy (RCT). Symptomatic tears in the tendon are frequently repaired surgically by rotator cuff repair (RCR) but little is understood as to how surgery may affect the repaired tissue. The central and peripheral nervous system play an important role in both tissue homoeostasis and tendon healing. We aimed to describe the key tissue changes that occur after rotator cuff repair in humans for the first time, including those relating to peripheral nervous system.
Methods
Paired rotator cuff tendon specimens were obtained from 8 patients undergoing rotator cuff repair using a novel ultrasound guided biopsy technique; the first specimen was taken immediately after repair and the second six weeks post operatively. The tendon tissue was histologically stained using Haematoxylin and Eosin, and immunohistochemically stained with primary antibodies visualised using 3,3-diaminobenzidine (DAB). Image analysis was performed blindly by 2 observers using Image-J to quantify the amount of DAB positive staining. Data was non-parametric in distribution and Wilcoxon matched-paired signed rank tests were carried out using SPSS with significance levels set at a minimum of $p<0.05$.
Results
Tendon vascularity (p-0.0039) and cellularity (p-0.0078) were significantly increased 6 weeks post operatively. The Glutaminergic system was significantly up-regulated at six weeks with an increase in Glutamate (p-0.0195) and changes in several related receptors ($p<0.05$). The nerve marker Protein Gene Product 9.5 (PGP9.5) was significantly increased at six weeks (p-0.01). There were no significant correlations between pain scores and the peripheral tissue markers.
Discussion/Conclusion
RCR results in significant tissue changes which involve the Glutaminergic system. These findings are novel and improve our tissue healing in RCT, and potentially provide novel therapeutic targets.

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THE USE OF BONE ADHESIVE AS A PRINCIPAL MODALITY FOR LONG BONE FRACTURE FIXATION

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Introduction:
The goal of fracture fixation is to restore anatomy, impart limb stability and ultimately permit predictable and uneventful fracture healing. Most long bone fractures are managed operatively. Current orthopaedic research is focussed on the development of improved implants and associated operative techniques. Adjuvant treatments to provide improved fracture fixation and promote bone healing are also becoming more widely adopted. Kryptonite osteoconductive adhesive (Kryptonite-OA), a commercially available injectable bone adhesive has been proposed as an alternative means of fracture fixation in long bones. It may have the potential to replace conventional internal fixation in closed fractures. However, there is no published evidence to support its sole use for fracture fixation.
Aim:
To test Kryptonite-OA as a primary tool for long bone fractures fixation.
Method:
A three point bending test and alignment jig were used to load four pig femurs, four Thiel-embalmed cadaveric humerii and one dry bone to failure. Bone specimens were then reconstructed in the alignment jig with Kryptonite-OA and re-fractured using the same test protocol. Bone specimens were dried as much as possible to aid adhesion. The rigidity, fracture strength and work to fracture for pre-fixation and Kryptonite-OA fixated were compared.
Results
All Kryptonite-OA fixated specimens behaved similarly with gross deformation occurring at the site of adhesive application. There were no sudden fractures observed within the adhesive, at the adhesive bone junction, or elsewhere in the bone specimens. Minimal resistance to loading was observed in all specimens. There were reductions of rigidity ranging from 66% to 94%., fracture strength ranging from 88% to 96% and work to fracture ranging from 77% to 99%. Minimal adhesive interdigitation into the cancellous bone was observed in all test samples.
Conclusion:
In its current formulation, Kryptonite-OA should not be used as the sole modality for long bone fracture fixation.

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LOCAL DECREASES IN CARTILAGE STIFFNESS LEAD TO CONTACT CHANGES INDICATIVE OF KNEE OSTEOARTHRITIS

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Cartilage stiffness decreases by at least 20% in early-stage osteoarthritis (OA) [1]. Subject-specific finite element models (Abaqus 6.11) were created to investigate the effects of decreased stiffness on the stresses and strains within the knee and some potential mechanisms by which OA may initiate.

Models combined geometry (derived from MRI) and load cases (derived from motion analysis) from three healthy subjects (six knees). The tibia, femur, tibial cartilage, femoral cartilage, and menisci were included. Three sets of models were created:

-Normal cartilage stiffness: E = 12 MPa.

-Globally-decreased cartilage stiffness: E of entire tibial cartilage and femoral cartilage decreased by 10% and 20%.

-Locally-decreased cartilage stiffness: Focal cartilage lesions modelled by decreasing E in locations of typical cartilage lesions [2] by 20%, 45%, and 85%.

Decreased cartilage stiffness increased displacement and strain magnitudes and decreased stress and contact pressure magnitudes in globally-decreased cartilage stiffness and locally-decreased cartilage stiffness models.

Globally decreasing cartilage stiffness had negligible visible effect on contact distributions. Perhaps larger decreases in cartilage stiffness are necessary for noticeable changes in contact distributions to be observed; cartilage stiffness modulus decreases of up to 80% have been reported in early-stage OA [1]. Also, in vivo cartilage matrix damage and lesions initiate in specific locations rather than affecting the entire cartilage structure; therefore, local decreases in cartilage stiffness should more accurately reflect the physiological conditions leading to cartilage lesions.

Locally decreasing cartilage stiffness visibly affected contact distributions. Increased displacement in areas of locally-decreased stiffness led to liftoff in other areas and shifted contact to areas of typical cartilage lesions. Cartilage has regional material properties, with stiffer cartilage in areas of frequent contact and loading [3]; shifting contact to areas of less-stiff cartilage could damage the cartilage and lead to degenerative diseases such as OA. Locally-decreased cartilage stiffness also increased strains within the cartilage. High strains could damage the fibres within the cartilage matrix, further decreasing cartilage stiffness and eventually leading to cartilage lesions and OA.

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[3] Athanasiou et al., 1994. J Orthop Res, 12(3):340-349.

P 40

AVAILABILITY OF HIGH IMPACT FACTOR ORTHOPAEDIC JOURNALS ON MOBILE TABLET DEVICES

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Background:

The expansion of mobile tablet technology has revolutionised the way research content can be accessed. Using mobile software applications, orthopaedic surgeons can access the latest research at the point of care. Digital journal applications offer a number of advantages over 'mobile-friendly' journal websites and traditional paper variants including the ability to download and store complete back issues, share articles and access additional media content.

Objective:

To quantify the number and availability of major international high impact factor orthopaedic journals on mobile tablet devices.

Method:

A specific search of the three major application stores was carried out specifically searching for the top twenty five journals as ranked by impact factor in the orthopaedic subject category of Journal Citation Reports (JCR) 2011. Apps that did not meet strict inclusion criteria were discarded.

Results:

28% (n=7) of the top twenty five impact factor orthopaedic journals were available on a mobile tablet. These were (in order of decreasing impact factor): ARTHROSCOPY (6), J BONE JOINT SURG BR (8), J SHOULDER ELB SURG (10), ACTA ORTHOP (15), J ORTHOP TRAUMA (16), CLIN J SPORT MED (18) and SPINE (19). All seven journals were available on the iOS platform whilst only one (ACTA ORTHOP J) was available on the Android platform. None of the orthopaedic journals were available on the Blackberry platform. Every journal application was available for free but required institutional or individual subscriber login to access full text content.

Conclusions:

There is a distinct lack of high impact orthopaedic journals available for mobile tablet devices. The overwhelming majority of those that have been developed are designed for the iOS platform. Further work needs to be done to improve equitable platform access whilst increasing overall journal availability on tablet devices.

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FUTURO OF BUCKLE OR BACKSLAB? A REVIEW OF THE MANAGEMENT OF PAEDIATRIC BUCKLE FRACTURES

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Many paediatric buckle fractures heal without complication in 3-6 weeks. Often this patient population receives a full Plaster of Paris backslab and fracture clinic follow-up.

Many children find backslabs uncomfortable and heavy. Splints can offer similar levels of support whilst allowing the patient to continue most of their daily activities without hindrance. Furthermore, splints can be safely removed at home without the need for a further hospital attendance.

As part of a Quality Improvement Project initiative at our Trust, we reviewed the evidence for treating simple paediatric buckle fractures with splints or backslabs. An audit of our Trust's current Emergency Department practise concluded that backslabs were used in most cases, with most children brought back to fracture clinic for review and cast removal.

As a result, we have developed and implemented a new algorithm for the management of simple buckle fractures that we hope will increase the numbers of children receiving splints, while still ensuring patient safety and appropriate referral for specialist Orthopaedic advice where relevant.

Working closely with Emergency Department and Orthopaedic teams, we aim to re-audit the outcomes of the children receiving splints in order to determine whether our protocol is being correctly followed.

Our ultimate goal is to determine whether the burden upon busy fracture clinics can be reduced, thereby reducing cost to the Trust and time pressures on patients and staff.

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MOBILE TABLET TECHNOLOGY AND PATIENT EDUCATION IN ORTHOPAEDIC SURGERY

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Introduction:

Patient education in orthopaedic surgery is a common and important intervention directly related to overall outcome. The expansion of mobile tablet technology and its rising use within the clinical setting means that medical software applications may play an increasingly important role in patient education. This novel technology allows surgeons to use tablet devices to deliver customisable patient education information to their patients in a preoperative setting with a potentially favorable cost-utility thus improving patient care and the patient experience.

Objectives:

This paper quantitatively assesses the availability of orthopaedic patient education applications and their respective features available for mobile tablet devices. Areas of potential future patient education application development and deployment in a clinical setting are identified.

Methods:

Evidence showed that the most popular tablet device amongst physicians in 2011 was the Apple iPad. A systematic search for mobile applications within the 'medical' category on the Apple App Store was conducted using keywords related to patient education and orthopaedic surgery. All applications which met the strict inclusion criteria were downloaded and evaluated.

Results:

A total of 76 applications were identified, which represented a total of 2.6% of all medical applications (n=2923) currently available. Only 10

of the applications identified by the search had a clear primary use as an orthopaedic surgery patient education app (0.34%). Qualitative analysis of these applications indicated that the following features were common to all orthopaedic patient education applications:
-Use of animations and video to explain procedures
-Ability to annotate images/videos
-Ability to export these via a range of media for the patient
-Information related a range of orthopaedic diseases and conditions in all areas of the body
Conclusion:
Despite the increase in mobile tablet usage at the point of care and the high number of medical applications available, there is a clear shortage of patient education applications. The primary method of patient education communication came from video animations which could be annotated by the surgeon whilst simultaneously explaining to the patient. This paper clearly highlights the need for more well-designed, informative orthopaedic patient education applications and systematic research into the impact of this new technology on overall outcome.

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USE OF DENOSUMAB AND ITS EFFECTS ON SERUM CALCIUM IN DIALYSIS PATIENTS

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Introduction: Denosumab (DN) is a human monoclonal antibody that inhibits osteoclast formation, function and survival thereby decreasing bone resorption. It is an effective therapy for osteoporosis in post-menopausal women. Its use in ESRD is yet to be explored. We report three haemodialysis and one peritoneal dialysis patient who received DN for osteoporosis.

Patient Age/Gender	Corrected calcium (mmol/L)		Phosphate (mmol/L)		ALP (U/L)	
	PRE	POST	PRE	POST	PRE	POST
Case 1	2.32	1.58	1.49	1.41	189	101
Case 2	2.65	1.98	1.74	1.13	98	92
Case 3	2.58	1.98	0.78	0.45	323	227
Case 4	2.33	1.97	0.98	0.62	336	196

PTH (pmol/L)		CTX (ug/L)		Total 25OH D (nmol/L)
PRE	POST	PRE	POST	PRE
16.3	NA	1.18	NA	NA
105	87.5	2.90	2.25	30.3
67.9	179	4.37	1.07	40.5
54.8	108.8	5.82	0.58	68.7

Case1: Seventy seven year old male on peritoneal dialysis administered a single dose of DN 60 mg subcutaneously (S/C). He developed symptomatic hypocalcemia requiring hospital admission for calcium supplementation. Patient developed long QT but there were no cardiac arrhythmias.
Case 2: Fifty year old female on haemodialysis, received DN 60 mg S/C. She was hospitalised for intravenous calcium supplementation.
Case 3: Seventy two year old female on daily haemodialysis had DN 60 mg. She developed asymptomatic hypocalcemia which was managed as an outpatient.
Case 4: Sixty four years old female on maintenance haemodialysis was given DN 60 mg dose. Asymptomatic hypocalcemia was noted and managed as an outpatient.
Conclusion: DN can cause symptomatic hypocalcemia in dialysis patients. Monitoring of calcium levels before, during and after the DN therapy can avoid this serious complication.

P 44

FAILURE IN THE APPLICATION OF FRAGILITY FRACTURE PREVENTION GUIDELINES; A PROSPECTIVE ASSESSMENT AT THE TIME OF ADMISSION WITH A NEW HIP FRACTURE

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Background: The estimated direct medical cost of fragility fractures to the UK healthcare economy was £1.8 billion in 2000, with the potential to increase to £2.2 billion by 2025. The majority of these costs are associated with hip fractures. We studied our hip fracture population on the day of injury in order to establish whether national guidelines are being followed and whether high risk patients are being identified and treated by primary and secondary care services
Methods: Data on a consecutive series of trauma hip fracture admissions were collected prospectively over a 14 month period at a central London teaching hospital. NICE, National Osteoporosis Guidelines Group (NOGG) guidelines and FRAX risk calculations were applied to patient data and further details were extracted from the local electronic trauma database.
Results: From 120 hip fractures a cohort of 94 could be assessed against national guidelines. The mean age of our population was 77.4 (range 51-89); 64 females and 30 males. 80% of the population were resident in London. The most common risk factor was smoking. 22% of patients had suffered a previous fragility fracture. FRAX 10 year probabilities of any fragility fracture or hip fracture were 14% and 7% respectively.
According to NOGG guidelines 45% of the study population required treatment, 35% fulfilled criteria for a DEXA scan and reassessment, and 20% needed no further management. In practice 27% were on treatment, 4% had undergone a DEXA scan but were not treated, and 69% were not on treatment and had not been investigated. In patients meeting intervention thresholds only 33.3% of those who should have been receiving treatment were receiving treatment in practice. This equated to 27% of the London population and 55.5% of patients from outside of London.
Conclusion: FRAX, in conjunction with NICE and NOGG guidelines, was able to identify 80% of our fracture population as intermediate to high risk on the day of fracture. Correct management however was only evident in only 33% of cases with a pattern of inferior care seen within London. There remains a lack of clarity over the duty of care in fragility fracture prevention.

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EFFECTS OF LUMBAR SPINAL OSTEOARTHRITIS (KELLGREN SCORE AND DISC SPACE NARROWING) ON SPINE AND HIP BMD IN OLDER ADULTS: THE EUROPEAN VERTEBRAL OSTEOPOROSIS STUDY.

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Men and women aged 50 to 85 years in 19 European countries participated in the European Vertebral Osteoporosis Study (EVOS) a population survey of vertebral osteoporosis. This analysis was undertaken to quantitate the effects of disc space narrowing (DSN), Kellgren Score (KS) and degenerative deformities on BMD (21 centres) in the evaluated vertebrae L2, L3 & L4.
The prevalence of Scheuermann's disease was evaluated in 14472 pairs of lateral thoracic and lumbar x-ray images obtained in the left side position, standard EVOS protocol (88% of the total were evaluable). In about 80% of these subjects, each vertebra from T4 to L4 was completely assessed for osteoporotic fracture, disc space narrowing, degenerative deformity and assigned a KS according to generally agreed criteria. Centres (n=6) were excluded from this analysis if their gradings for degenerative disease were not completed (due to funding constraints or poor quality images), leaving 3895 subjects from 15 centres remaining in this analysis (1104 also with a second BMD at 3

years). The effects of DSN and KS (averaged from L2- L4; scale 0-4) on baseline DXA cross-calibrated BMD was evaluated separately for 3 measurement sites in linear models that included the effects of age, sex, an interaction between age and sex, Scheuermann's, vertebral osteoporotic fracture (both: yes/no) and investigational centre.
DSN in L2-4 was observed in 5% and the mean KS was 2. Degenerative deformities were rare (8/3895 subjects). Increasing KS increased L2-4 BMD, but the effect was not significant until KS >2. As KS rose from 2 to 3 there was an increase of 0.1 g.cm-2 with a further rise of 0.1 g.cm-2 as it rose from 3 to 4. DSN independently raised BMD by a further 0.15 g.cm-2. The effects of KS were similar but 30% as large on femoral neck and trochanter BMDs where DSN had no effect. In 1104 subjects with repeat BMD, only the most severe KS (grade 4) and DSN were associated with further increases in L2-4 BMD.
The substantial effects on lumbar spine BMD of local degenerative disease did not differ significantly between European centres.

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EFFECTS OF OF SCHEUERMANN'S DISEASE (ADOLESCENT EPIPHYSITIS) ON SPINE AND HIP BMD IN OLDER ADULTS: THE EUROPEAN VERTEBRAL OSTEOPOROSIS STUDY.

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Men and women aged 50 to 85 years were recruited from 36 centres in 19 European countries into the European Vertebral Osteoporosis Study (EVOS) a population survey of vertebral osteoporosis. This analysis was undertaken to resolve uncertainty concerning the effect of Scheuermann's disease (SD) on adult bone mineral density, previous work having been based on small case-control series.
The prevalence of SD was evaluated in 14,472 pairs of lateral thoracic and lumbar x-ray images obtained in the left side position using the standard EVOS protocol (88% images evaluable). The criteria for SD included the presence of one or more Schmorl's nodes and/or irregular vertebral endplates in combination with one or more of three other signs to make a minimum of three positive signs. The minor signs were: increased thoracic kyphosis, decreased intervertebral space and wedged vertebrae. Less frequent but pathognomonic was the Edgren-Vaino sign. Among the 14472 subjects, 3472 had BMD measured in the lumbar spine (L2-L4) and 3900 in the femoral neck and trochanter, which was cross-calibrated with the European Spine Phantom. The effect of SD on BMD was evaluated separately for the 3 measurement sites in a linear model that included the effects of age, sex, an interaction between age and sex and investigational centre. The modelling was undertaken both including and excluding subjects diagnosed with osteoporotic vertebral deformities.
The overall prevalence of Scheuermann's disease was 6.8% in men and 6.9% in women, and located most frequently in the mid part of the thoracic spine. Increased thoracic kyphosis was found in nearly half of cases. Ssheuermann's slightly increased L2-L4 BMD in younger subjects but there was a larger effect of age, with a faster apparent rate of loss (p=0.008) so that older subjects had comparatively reduced BMD. No significant effects were seen on hip BMD. Excluding subjects with osteoporotic fractures had no effect on these results.
We conclude that Scheuermann's disease is not significantly associated with adult spinal osteoporosis, but is up to half as prevalent as spinal fragility fracture in those over 50 years. BMD measurement can be helpful in cases of radiological uncertainty.

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BONE LINING CELL DEDIFFERENTIATION: A POTENTIAL NEW METHOD TO STUDY BONE REGENERATION

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Bone formation depends on the activity of bone matrix producing osteoblasts which counteract bone destruction by osteoclasts throughout life to maintain bone homeostasis. Whilst it is known that active osteoblasts are recruited from an osteoprogenitor cell pool of mesenchymal origin to contribute to bone repair, it is currently unclear whether fully differentiated bone lining cells (BLC, a type of mature osteoblast) can dedifferentiate to contribute to bone formation upon injury in mammals. In this project, we will uncover the dedifferentiation potential of BLCs and their ability to contribute to bone repair in the mammalian system using murine models of fracture repair and osteoporosis. In osteoporosis, bone loss might be partially caused by a reduced potential of BLCs to contribute to bone anabolism whilst enhanced osteoblast activity in fractures would speed the repair process. We will identify the molecular processes leading to BLC dedifferentiation by genome wide expression analysis and characterize the impact of dedifferentiated BLCs on the maturation of bone degrading osteoclasts, which strongly rely on osteoblast maturation signals to reach functionality. Finally, this study aims to increase bone formation by modulating the developmental program of BLCs in an osteoporosis model of bone repair.

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EVALUATION OF ENHANCED SKELETAL TISSUE FORMATION BY DUAL GROWTH FACTOR RELEASE IN A NOVEL EX VIVO ORGANOTYPIC MODEL

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Development of alternative tissue engineering strategies to augment bone repair and regeneration is dependent on suitable animal models. Ex vivo model systems represent a promising alternative to simple in vitro cell systems or complex, ethically unsound in vivo models. We have recently developed a 3D ex vivo culture system of embryonic chick femora, adapted in this study to investigate effects of novel gel scaffolds containing growth-factor releasing microparticles and skeletal stem cells on bone regeneration.
Alginate/bovine ECM gel scaffolds were combined with PLGA/Triblock (10-30% PLGA-PEG-PLGA copolymer) microparticles; fast-releasing large microparticles (50-100µm) loaded with 15ng/ml TGF-beta3, or slow-releasing small microparticles (20-30µm) loaded with 100ng/ml BMP-2. Human serum albumin (HSA) was used as a control loading protein. Gel scaffolds were also loaded with human adult Stro-1 selected bone marrow stromal cells (BMSCs). Following calcium chloride cross-linking, 2mm cylindrical gel segments were created and placed into 2mm central segmental defects in embryonic day 11 chick femurs. Femurs were placed into organotypic cultures for 10 days in basal media, and analysed by micro-computed tomography, and histologically for proteoglycan (Alcian blue) and collagen production (Sirius red), as well as expression of bone and cartilage markers (collagen I/II).
Alginate/ECM gels loaded with HSA control protein, with or without BMSCs, displayed minimal matrix or expression of bone or cartilage markers. Addition of microparticles releasing BMP-2 alone, or TGF-beta3 and BMP-2 combined, increased Sirius red-stained matrix and collagen I / II expression within the gels, although the matrix was disperse and disordered. Addition of BMSCs into the gels containing BMP2-releasing microparticles resulted in deposition of a more structured bone matrix into the gel. This effect was significantly enhanced with addition of BMSCs to gels containing combined dual TGF-beta3/BMP-2 releasing microparticles, with formation of a highly-structured bone matrix within the gel.
This study demonstrates the successful use of the organotypic chick femur culture system as a high-throughput test model for scaffold/cell/growth factor therapies for regenerative medicine. Temporal release of dual growth factors, combined with BMSCs, improved formation of a highly structured bone matrix compared to single release modalities.

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VALIDATION OF THE RADIOGRAPHIC UNION SCALE IN TIBIA AND LANE AND SANDHU SCORING SYSTEM FOR FRACTURE HEALING IN SMALL ANIMAL MODEL

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Introduction

The validity and reliability of fracture healing measurement are essential for pre-clinical study. It has been reported that the Radiographic Union scale in Tibia (RUST) has an excellent inter-rater reliability. However, to date, the RUST score has not been evaluated in a pre-clinical model. Therefore, the objectives of this study were (1) to determine the intra- and inter- observer of RUST and Lane and Sandhu scoring system from orthopaedic surgeons and researchers in the orthopaedic field and (2) to demonstrate the limits of agreement of both scoring system using the Bland-Altman plot.

Method

Thirty sets of anteroposterior and oblique radiographs of different stages of tibial shaft fractures in a pre-clinical model treated with external fixation were selected. Inter- and intra- observer reliability was determined from the scores of six observers, including three orthopaedic surgeons and three orthopaedic researchers (one laboratory researcher, one bioengineer and one physiotherapist). The intra-class correlation coefficient (ICC) was used to determine Inter- and intra-observer reliability. The Bland-Altman Plot was used to demonstrate the limits of agreement of both scoring systems.

Results

The overall inter-observer agreement for general impression was moderate (0.58, 95%C.I. = 0.49-0.65). The intra-observer agreement for surgeons was better than for non-surgeons. The overall inter-observer agreement of RUST score was almost perfect (0.81, 95%C.I. = 0.72- 0.89). There were strong agreement in non-surgeon raters and almost perfect in surgeon raters. Similarly, the overall inter-observer agreement of the Lane and Sandhu score was almost perfect (0.88, 95%C.I. = 0.81 - 0.93). All of the raters had almost perfect agreement using the Lane and Sandhu score. Both scoring systems had a very good agreement showing by the Bland-Altman Plot.

Discussion

The RUST and the Lane and Sandhu score were demonstrated to be superior to general impression in the pre-clinical setting. These scores were especially useful if non-surgeons were assessing the healing process on x-ray. Both scores were comparable with excellent reliability.

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IS THERE ANY RELATIONSHIP BETWEEN OXFORD HIP SCORES AND BLOOD METAL ION CONCENTRATIONS FOLLOWING METAL-ON-METAL HIP REPLACEMENT?

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Background

MHRA guidelines recommend measuring blood metal ion concentrations in all symptomatic patients with metal-on-metal (MoM) hip replacements. Regarding asymptomatic patients, blood tests are recommended in large head MoM THRs but not in small head THRs or in hip resurfacings. This study aimed to determine the relationship between the Oxford hip score (OHS) and blood metal ion concentrations.

Patients and Methods

All patients undergoing a MoM THR or a Birmingham hip resurfacing (BHR) with blood metal ion concentrations measured at this centre up to April 2013 were included. Patients were eligible for inclusion if they had completed an OHS questionnaire (0% asymptomatic to 100% worst-possible joint) at the time of blood sampling and had a unilateral MoM hip bearing in-situ for more than 12 months that had not been revised. Linear regression analysis was performed to define an equation predicting blood metal ion concentration from OHS.

Results

There were 518 eligible patients (330 THR and 188 BHR). Mean age (range) at surgery was 60.1 years (21.8-89.1) for the THR group (50% male) and 49.0 years (14.6-75.0) for the BHR group (54% male). THR median (interquartile-range) blood metal ion concentrations were 2.06 microg/l (0.83-3.71) for cobalt and 1.25 microg/l (0.83-2.03) for chromium. BHR median (interquartile-range) blood metal ion concentrations were 1.00 microg/l (0.71-2.06) for cobalt and 1.61 microg/l (0.71-2.65) for chromium. Metal ion levels greater than the MHRA thresholds of 7 microg/l were found in 33 (9.4%) THR patients and 21 (10.7%) BHR patients. THR median (interquartile-range) OHS was 10.4% (2.1-31.5) and for the BHR was 35.4% (12.5-56.2). The linear regression lines for THR and BHR groups showed no significant relationship between OHS and blood cobalt or chromium concentrations with R-squared always less than 0.01.

Conclusions

Pain and disability measured with the OHS was not a predictor of blood metal ion concentrations for MoM THRs and BHRs. The OHS is therefore not a good surrogate marker for blood metal ion concentrations in patients with MoM bearings.

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HISTOLOGICAL AND CLINICAL EVALUATION OF FAILED SHOULDER SURFACE REPLACEMENT IMPLANTS

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Introduction: Cementless surface hemi arthroplasty of the shoulder has achieved popularity as an alternative to conventional arthroplasty for treatment of painful shoulder arthritis. The aim of this study was to investigate the hypothesis that increased distal stem fixation resulted in stress shielding causing decreased bone formation, viability and osseointegration, leading to the failure of uncemented resurfacing implants retrieved at revision.

Methods: Twelve failed resurfacing shoulder implants retrieved from patients were assessed. Nine female and 3 male patients with a mean age of 63(range, 42-75 years) were investigated. Retrieved specimens were processed for undecalcified histology. Prior to analysis, implants were divided into four regions; under the cup, proximal stem, mid stem and distal stem. Image analysis techniques were used to quantify bone-implant contact, bone area and bone viability within these four regions adjacent to the implant.The Spearman's rank coefficient was used to assess correlations between pairs and a Mann Whitney-U test used to determine significant differences between regions. In all cases p values <0.05 were considered significant. All implants were also examined using Backscattered Scanning Electron Microscopy.

Results: Results showed significantly increased bone contact under the cup of the implants (20.12%±15.38%), when compared with measurements obtained from other regions of the stem ($p<0.05$ in all cases). In addition, significantly increased bone area was measured beneath the cup and adjacent to the proximal region of the stem when compared with the mid and distal stem ($p<0.05$ in all cases). When results for percentage bone viability were compared, viability was significantly higher in the sub cupola region than the distal stem ($p=0.038$).

When all implants and regions were combined, results showed a mean bone-implant contact of 15.82%±14.99%, a mean bone area of 0.11±0.11 mm2 and a mean viability of 63.58%±18.54%. No significant correlations between bone viability, bone contact and bone area were found.

Conclusions: This study showed that in failed uncemented shoulder implants, greater osseointegration, bone formation and bone viability occurred beneath the cup of the implant when compared with the stem. These results suggest that stress shielding under the cup may not be the most important contributor to implant failure.

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NATURAL HISTORY OF PAIN FOLLOWING UNICOMPARTMENTAL KNEE REPLACEMENT

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Medial unicompartmental knee replacement (UKR) is an alternative to total knee replacement for osteoarthritis and has been shown to have better functional outcomes in clinical studies. However the revision rate is higher and revisions are often attributed to unexplained pain. Proponents of UKR suggest that this pain is likely to improve over the first postoperative year, but this has not been demonstrated empirically. The aim of this study was to define the natural history of pain following UKR, and to determine the factors affecting incidence of, and recovery from, postoperative pain.

191 knees in 183 patients underwent UKR (Oxford UKR, Biomet, Bridgend UK) in a single institution. Mean age was 65.2 years (36.6-86.5) and 52% of knees were in females. Oxford Knee Scores were recorded on each patient preoperatively, at six weeks and one year post-op. At each time, pain was classified as mild/none, moderate, or severe. Pain was classified as 'explained' if an obvious cause was present (eg infection or trauma). Where no other cause was found, pain was classified as 'unexplained'. Patient factors (body mass index (BMI), age, gender) and surgeon grade were recorded.

At six weeks, pain was severe in 7/191 knees (3.7%) and moderate in 51/191 (27.2). At one year pain was severe in 6/191 (3.1%) and moderate in 27/191 (14.1%). 73/191 (38%) reported pain at either time point; 56/73 (77%) were unexplained. Pain improved between 6 and 52 weeks (one way ANOVA, $p<0.05$ for all outcome measures, across all time intervals), regardless of whether it was explained or not. The incidence of unexplained pain was unaffected by age, BMI or surgeon grade. Women were more likely to experience unexplained pain than men (Chi Squared test, $p=0.02$). Neither age, gender, BMI nor surgeon grade affected the progression of pain beyond 6 weeks.

This study demonstrates that unexplained pain after UKR is likely to improve in the first postoperative year. Whilst women are more slightly more likely to experience unexplained pain at 6 weeks, neither age nor BMI affected the incidence of pain. Neither age, gender nor BMI affected the progression of this pain beyond six weeks.

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PRESSURE DISTRIBUTION BENEATH THE TIBIAL TRAY IN MOBILE-BEARING UNICOMPARTMENTAL KNEE REPLACEMENT DURING NORMAL AND ABNORMAL LOADING

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Aims: Cementless Unicompartmental Knee Replacement (UKR) provides excellent radiological and functional outcomes at one to five years with no increase in complications compared to the cemented implant. However, a small number of cases have been identified which demonstrate early tibial component subsidence into valgus before becoming well-fixed. These appearances may be due to impingement of the mobile bearing on the lateral wall of the tibial component.

Methods: An in vitro model of cementless UKR was constructed using tibial components implanted into commercially available polyurethane blocks (Sawbones, inc.). These have been validated for the testing of orthopaedic implants and have a density equivalent to normal cancellous bone. Tests were performed using a material test machine (Dartec Ltd., Stourbridge, UK). Two separate test protocols were performed. The first represented normal anteroposterior bearing movement during a simulated sit-to-stand activity. The second represented bearing impingment on the lateral tibial wall. Measurements were recorded using thin-film pressure sensors (Tekscan Inc., South Boston) under the tibial component.

Results All tests demonstrated an area of increased pressure around the edge of the prosthesis, corresponding to the ridge around the cement pocket of the tibial component. Mean rim pressure was 632.0kPa (SD 68.5), compared with 104kPa (24.1) in the interior. During normal loading, there was no significant difference between pressures medial and lateral to the keel (429.1 (66.3) v 374.6 (79.3), $p=0.23$). Impingement of the bearing against the wall has a profound effect on loading, resulting in a mean pressure lateral to the keel of 972.3kPa (SD 93.5).

Conclusions: Lateral wall impingement leads to dramatic changes in pressure distribution beneath the tibial tray and may explain valgus subsidence. Avoidance of excessive medialiasation of the tibial tray may avoid this phenomenon.

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APATITE-FORMING ABILITY OF THE NEW TI-NB-SN ALLOY WITH LOW YOUNG'S MODULUS VIA ANODIC OXIDATION AND HOT WATER TREATMENT

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Ti-6Al-4V alloy is widely used as orthopedic implants. However, stress shielding occurs after uncemented total hip arthroplasty because its Young's modulus (110 GPa) is much higher than that of human femur. We developed a new Ti-Nb-Sn (TNS) alloy which Young's modulus is less than 50 GPa. On the other hand, the surface modification which has a more secure bone-bonding ability is desirable for orthopedic implants. We focused on the anodic oxidation (AO) adding subsequent hot water treatment (HW). This treatment had a good ability of hydroxyapatite (HA) formation on the commercially pure titanium (Cp-Ti) in simulated body fluid (SBF). In this study, we investigated the HA formation on the TNS alloy surface which was treated by AO + HW treatment.

We prepared discs (10 mm diameter and 2.0 mm thickness) made with TNS alloy and Cp-Ti. These discs were anodized in 2M acetic acid followed by the immersion into 15ml of distilled water at 80 degrees for 48 hours. Each disc was soaked in 25ml of SBF at 36.5 degrees for 7 days. The surface morphology was observed by FE-SEM. We added XRD and XPS analysis. We used Cp-Ti disc treated by AO + HW as a positive control.

After soaking in SBF, in SEM image, HA did not form on the surface of materials treated by only AO, but HA nucleation was observed by subsequent HW treatment in both alloys. In XRD, the anatase phase could be developed by AO + HW treatment. In XPS analysis, the intensity of O1s spectra derived from OH- increased after HW treatment.

AO in acetic acid solution could develop an amorphous titania which did not have a potential of HA formation. XRD patterns indicated that subsequent HW treatment could convert the amorphous into the anatase which could develop HA nucleation. In XPS analysis indicated that Ti-OH groups increased after subsequent HW treatment. The surface of the materials were negatively charged in SBF, and then positively charged Ca2+ ions bond, finally HA nucleation was developed.

In conclusion, AO combined with HW treatment could make HA nucleation on the surface of TNS alloy.

P 55

GLUCOSE CERAMIDE SYNTHASE INHIBITORS PREVENT OSTEOCLAST ACTIVATION AND LIMIT MYELOMA INDUCED OSTEOLYTIC LESIONS

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Glycosphingolipids (GSL) are essential structural components of mammalian cell membranes and lipid rafts that exert pleiotropic effects on cell survival, proliferation and differentiation. Cancer associated GSL have been shown to promote tumor growth, angiogenesis and metastasis; however their role in osteoclast (OC) activation and the development of osteolytic bone diseases such as multiple myeloma are not known.

We investigated the hypothesis that GSL contribute to OC activation and inhibitors of GSL biosynthesis would antagonise GSL-dependent osteoclastogenesis.

Exogenous addition of GM3, the prevailing GSL produced by myeloma plasma cells, synergistically enhanced the ability of the pro-osteoclastogenic factors RANKL and IGF-1 to induce the maturation of OC in vitro. However, these effects were inhibited by the glycosphingolipid synthesis inhibitor N-butyl-deoxynojirimycin (NB-DNJ). In vivo administration of GM3 increased OC numbers and

activity; this effect was reversed by treatment with the iminosugar agent NB-DNJ. NB-DNJ prevented OC development and activation by disrupting RANKL-induced localisation of TRAF6 and c-Src into lipid rafts thus attenuating MAPK signalling.
Therapeutic potential of NB-DNJ was proven by employing the 5TGM1 multiple myeloma mouse model where we were able to demonstrate a significant improvement in bone parameters compared to the PBS treated mice.
These data demonstrate a novel role for tumor-derived, as well as of de novo-synthesised GSL, in OC differentiation and activation and suggest that glycosphingolipid synthesis inhibitors, such as the clinically approved NB-DNJ, may be beneficial in reducing OC activation and bone destruction associated with multiple myeloma.

P 56

HYPERPARATHYROIDISM SECONDARY TO TREATMENT WITH BISPHOSPHONATES, ABOUT 4 CASES

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Bisphosphonates have seen their place considerably increased of bone diseases. By their action on the inhibition of bone remodeling, they may be responsible for an increase parathyroid hormone (PTH) compensation. Objectives: Additional tests are needed to discriminate various differential diagnoses and ensure its long-term normalization.
1: Patient aged 65. Followed for pancreatic neoplasia in 2009, treated with chemotherapy. Nodular goiter in 2009. Hepatitis C diagnosed in 2010.reveiced alendronate for densitometric osteoporosis. Biological assessment bone was correct. Biological control achieved during follow-up (10 months), found Hyperparathyroidism at 189 ng / l, normal vitamin D. bisphosphonate treatment was stopted. Control of parathyroid hormone after 15 days found a rate of 95 ng / l.
2: Patient 76 years old, menopause at the age of 50 years, unsubstituted. Followed for hypothyroidism in Levothyrox 100 ug / day and. she received alendronate for postmenopausal osteoporosis, took 18 months.PTH WAS AT 117.8 pg / ml. The rest of the bone were normal. The conduct was to repeat the PTH assay to 3 weeks of discontinuation of osteoporosis as well as calcium, phosphorus a treatment. The result was, respectively, 66.1 pg / ml, 96 mg / l and 34 mg / l. Calcium excretion of 24 to 104 mg/24 h and 616 mg/24 phosphaturia.
3: Patient aged 71, menopause at the age of 40 years. History of fracture of the right forearm to slip fall. Followed for osteoporosis since 2007, postmenopausal, treated with alendronic acid. BMD in the middle of sequence found a significant decrease in bone mineral density at the lumbar spine and the left forearm. PTH was at 72.38 ng / l, 25 (OH) vitamin D to 39 ng / ml.Control of parathyroid hormone after 15 days stoping treatment was 52 ng / l.
4: Patient 79 years old. Followed for osteoarthritis. Alendronic acid in 5 years. Addressed for osteoporosis assessment. PTH was at 108.3 pg / ml. 25 (OH) vitamin D2-3 to 28.6 ug / l.The results after stoped treatment; PTH 65 pg / ml.
Discussion and conclusion:
Our four observations support cases arising under bisphophosphonates hyperparathyroidism.

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ASSOCIATION ENTEROPATHY EXUDATIVE AND DERMATOMYOSITIS, A CASE REPORT

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Introduction
The protein-losing enteropathy is a rare condition. It is a violation of the lymphatics of the submucosa. It can be associated with autoimmune diseases, typically, celiac disease and lupus. We report a case.
Observation
Patient aged 16. No medical history. Admitted to diffuse myalgias, muscle weakness predominant in belts with erythro-bilateral eyelid edema. Peripheral edema pitting and diarrhea liquidiennes. CPK increased at 294UI / l, hypoalbuminemia at 24.5 g / l, increasing the clearance of alpha 1-antitrypsin 55 ml/24 hours, the anti ac tranglutamine, IgA <12 U / ml, alpha 1 antitrypsin <1.70 U / ml. Myogenic on electromyography. Cardiomyopathy echocardiography with cardiac liver expansion of the inferior vena cave and hepatic veins. The diagnosis of dermatomyositis associated with protein-losing enteropathy was made. The treatment was, corticosteroids 1 mg / Kg / day. Evolution: Regression signs of dermatomyositis and parallel signs of enteropathy (normalization of serum albumin and decreased clearance of alpha 1 anti-trypsin).
Conclusion
The protein-losing enteropathy is a rare condition. Cases of association with CREST syndroma and cryoglobulinemia sjogren have been reported. An association with dermatomyositis, illustrated by our case is less known.The association of entheropathy exudative with certain autoimmune diseases raises the question of a possible immune involvement in the etiology of the disease.

P 58

ENHANCED OSTEOGENESIS ON PEEK POLYMER USING OXYGEN PLASMA TREATMENT

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Polyetheretherketone (PEEK) is a thermoplastic polymer that is predominant in spinal surgery as the material of choice for spinal fusion cages, and is also used for bone anchors, cruciate ligament interference screws, and femoral stems. It has the distinct advantage of having similar mechanical properties to bone, but its clinical application as an implant material is limited by a lack of bioactivity. This project aims to create a PEEK surface capable of osseointegration using a surface modification technique known as oxygen plasma treatment.
PEEK surfaces were injection molded, washed and then treated in a plasma chamber in the presence of oxygen for up to ten minutes. Surfaces were characterised using atomic force microscopy (AFM), scanning electron microscopy (SEM), water contact angle measurements and X-ray photo-electron spectroscopy (XPS). Human bone marrow cells were cultured on the surfaces for six weeks then stained with alizarin red and assessed for surface coverage and calcium production using high resolution scanning, polarized light microscopy, and image analysis software.
Water contact angle measurements show that after plasma treatment, the surfaces become very hydrophilic, before developing a meta-stable state after eight weeks. AFM and SEM showed destruction of the nano-pits at treatment durations longer than two minutes. XPS detected a progressive increase in the atomic proportion of oxygen at the surface with increasing plasma treatment duration. There was significantly more alizarin uptake (and hence calcium production) on the plasma treated PEEK compared to the untreated PEEK surfaces ($p < 0.05$).
These results show that oxygen-plasma treatment can increase surface coverage and calcium production on PEEK surfaces and may improve long term osseointegration of PEEK implants when used in clinical practice.

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INCREASED INTERFACIAL BONE CONTACT USING TITANIUM COATED NANO-PATTERNED IMPLANTS ON RABBIT TIBIAE

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Titanium is a popular material used for bone implants in orthopaedic and dental surgery. Surface modification of titanium is often used to alter the topography or chemistry of the surface with the aim of improving ossseointegration and long term functionality of the implant. This project compares a new method of surface modification (nano-patterning) with a current clinical standard (grit-blasting and acid-etching (GAE)).
Titanium discs were blasted with aluminium oxide and etched in sulphuric and acetic acid. Injection molded nano-pitted polycarbonate

discs (with two different nano-patterns) were coated in titanium by evaporation. The topography and chemistry of the discs was assessed using atomic force microscopy (AFM), scanning electron microscopy (SEM), water contact angle measurements, and X-ray photo-electron spectroscopy (XPS). The discs were plated onto a flattened area on the tibiae of 12 rabbits and were removed after 4 and 8 weeks for histological assessment. Samples were stained with methylene blue and basic fuschin and three cross-sections of each disc were analysed for the bone implant contact (BIC) ratio.
AFM demonstrated the difference in the ordered square (SQ) and randomly disordered (RAND) nanopatterns. The GAE surfaces exhibited micro-scale roughness whereas the titanium coated SQ and RAND surfaces had nano-scale roughness and preserved nanopits (approx. 100nm deep). Water contact angle measurements showed the surface had comparable wettability and XPS demonstrated similar chemical compositions with the exception that the GAE surfaces contained 6.8% aluminium.
Histological samples analysed at 4 weeks showed a BIC ratio of 36% for GAE, 56% for SQ, and 48% for RAND. At 8 weeks, the BIC ratio was 52% for GAE, 80% for SQ, and 72% for RAND. There was 54% more BIC in the nanoscale topography (SQ) compared to the current clinical standard (GAE) ($P < 0.05$).
This project demonstrated there was a significant increase in interfacial bone to implant contact when using a nano-scale topography incorporating a square array of nanopits compared to conventional grit-blasted acid-etched micro-scale topographies This enhancement of BIC may reduce long term loosening of orthopaedic or dental implants due to mechanical and biological attrition at the interface.

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THE PPARGAMMA LIGAND BADGE REDUCES TUMOUR BURDEN AND INCREASES BONE MARROW ADIPOSITY IN MULTIPLE MYELOMA IN VIVO

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Interactions between myeloma (MM) cells and cells of the bone marrow microenvironment promote tumour growth, survival and osteolytic bone disease. A better understanding of these interactions is essential to identify new therapeutic approaches for this fatal malignancy. The role of osteoclasts and osteoblasts are well studied, however the contributions of other cell types, including bone marrow adipocytes, are poorly understood. We hypothesized that a change in adipogeneis within the bone marrow microenvironment may play a role in MM development. Peroxisome proliferator-activated receptor gamma (PPARgamma) promotes adipocyte differentiation, and the aim of the study was to target PPARgamma in vitro and in vivo. C57Bl/KaLwRij mice were treated with Bisphenol-A-DiGlycidyl-Ether (BADGE, 30mg/kg daily i.p.), a compound reported to regulate PPARgamma activity and adipogenesis, or vehicle, and inoculated with 5TGM1 myeloma cells. BADGE treatment significantly decrease tumour growth rate (as measured by serum IgG2bK concentrations, $p<0.01$), reduced tumour burden within the bone marrow by 55% ($p<0.001$), and increased MM cell apoptosis of MM-bearing mice, compared to vehicle. No significant difference in trabecular bone volume was found, however a significant increase in the number of bone marrow adipocytes was observed ($p<0.05$). No significant difference was found in non-tumour mice. In support of an increase in adipogenesis, BADGE treatment increased PPARgamma mRNA expression ($p<0.01$), downstream targets adiponectin ($p<0.001$) and C/EBPalpha ($p<0.01$) in the bone marrow of MM-bearing mice, as compared to vehicle treated mice. In vitro studies demonstrated BADGE dose-dependently decreased MM cell viability, with no effect on viability of osteoblasts or bone marrow stromal cells. This direct anti-tumour effect of BADGE in MM cells was associated with a dose dependent increase in phospho-AMPK and Sirt1 protein expression, upregulation of osteolineage markers, RUNX2 and osteocalcin mRNA expression. Blocking PPARgamma using known antagonists together with BADGE does not change the anti-tumour effect of BADGE treatment alone in MM cells, suggesting that BADGE acts at least in part through PPARgamma-independent mechanisms in vitro. Overall, our studies demonstrated a striking reduction in tumour burden in response to BADGE, associated with an increase in bone marrow adiposity. Elucidating the mechanism of action of anti-myeloma effect of BADGE will reveal new therapeutic approaches for treatment of myeloma.

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WNT10B EXPRESSION AND FATTY DEGENERATION IN SUPRASPINATUS MUSCLE OF THE RABBIT AFTER SURGICAL REPAIR.

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The aim of this study is to analyze wnt10b expression in SSP muscle after surgical repair and the timing of surgical repair which can prevent fatty degeneration using rabbit model. First, we investigated the gene expression and histological analysis in the rabbit rotator cuff tear model. Next, we assessed rabbit rotator cuff repair model.
Rotator cuff tear model: SSP tendons were detached from the greater tuberosity and wrapped with a polyvinylidine fluoride (PVDF) membrane to prevent spontaneous reattachment (Detached side). Sham operation was performed in the other side (Control side). Rabbits were euthenized at 7, 14, 21, and 42 days. Rotator cuff repair model: SSP tendons detached and wrapped with PVDF membrane were reattached to the greater tuberosity at 7, 14 and 21 days after detachment. Rabbits were euthenized at 42 days after initial detachment. Total RNA extracted from SSP muscle was subjected to gene expression analyses by quantitative RP-PCR. The frozen section slides were stained with the Oil red-O solution. Oil red-O staining on the section was eluted by 100% 2-propanol and quantified by spectrophotometer at 510 nm.
In rotator cuff tear model, the Oil red-O staining showed oil fatty degeneration. The quantification of oil deposit exhibited significant increase at 7, 21 and 42 days after detachment.Depletion of wnt10b and elevation of PPAR gamma and C/EBP alpha mRNA expression in the detached muscles were observed.In rotator cuff repair model, Oil red-O staining of muscles reattached after 7, 14 and 21 days post-detachment showed a significant increase as compared with control side. The difference in the extent of Oil red-O showed a trend of increase in the time dependent manner of post-detachment. The gene expression of Wnt10b also decreased in the time dependent manner of post-detachment. The difference was significant in the detached muscles reattached after 14 and 28 days post-detachment.
Wnt10b in rotator cuff repair model showed the lower level of gene expression in experiment with the longer period between detachment and reattachment. The recovery of wnt10b expression and fatty degeneration may become irreversible in proportion to the period of time before surgical repair.

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THE FUTURE PROJECTION OF TOTAL KNEE REPLACEMENT IN THE UNITED KINGDOM

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Total knee replacement (TKR) is an effective intervention in patients with end-stage knee osteoarthritis. Although cost-effective, the future TKR cost burden to healthcare providers will be substantial. For the United Kingdom, there are no recently published projections for the number of TKRs over the next 25 years.
We produce projected estimates for future TKR counts over 25 years, based on a large population-based dataset, coupled with information from other sources. We report TKR counts by age and gender, and further stratify TKR projections by body mass index (BMI).
We used the UK General Practice Research Database (GPRD), identifying all subjects (N=23260) undergoing TKR between 1991 and 2010 who have a pre-operative recording of BMI. We used age-gender stratified person time and further split this by estimated categorical BMI by taking BMI proportions from the Health Survey for England as a proxy population-level BMI breakdown over the same time period.

Using a log-linear (Poisson) rates model, we applied TKR rate estimates to UK population forecasts from the Office for National Statistics, projecting TKR counts forward in time from 2010 to 2035. Estimated future changes in BMI population proportions were modelled linearly, but with an inverse hyperbolic tangential smoother to avoid invalid BMI category proportions in future years. Different projections scenarios were modelled and appropriate sensitivity analyses were carried out.

Using TKR rates fixed at 2010 levels, stratified by age, gender and BMI, we projected the number of TKRs in 2035 to be 118,000 (50,000 men; 68,000 women), an increase of just over 50% on 2010 levels. Analyses of the results by age and BMI produced some interesting temporal changes, with faster growth in the obese. When projecting by using the annual increase in TKR rates as estimated by the log-linear model, we see a dramatically higher number of TKRs in 2035 (approximately 1,200,000).

This work estimates future TKR counts from population-level incidence data but using a novel combination and structuring of additional data sources. In the absence of reliable TKR forecasts from government or healthcare organisations, these estimates may be of use as a guide to future levels of TKR surgery.

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A CLINICAL AUDIT ON NUTRITIONAL ASSESSMENT OF PATIENTS WITH FRACTURE NECK OF FEMUR IN AN ORTHO-GERIATRIC UNIT IN A DISTRICT GENERAL HOSPITAL

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Back ground

It is established that about 60% of patients with hip fracture are malnourished on admission to hospital. Evidence indicate that their morbidity, length of stay and mortality increase with malnutrition. Therefore it is of paramount importance to identify patients with increased risk of malnutrition and intervene at the point of admission to hospital. NICE recommends screening of nutritional status of all patients on admission to hospital.

Objective

We set out to audit our practice of nutritional assessment and subsequent management of patients at risk, in our Ortho-geriatric unit.

Method

We analysed 50 random case notes retrospectively against NICE guidance 'Nutritional Support in Adults - QS24'. 45 were analysed using an audit pro-forma. 5 cases were discarded due to non availability of the relevant documents.

Results

Only 32 (71%) patients had Malnutrition Universal Screening Tool (MUST) score calculated and in 50% of those the documentation of MUST score calculation was poor. Out of those 32, 20 (62%) were identified as low risk, 5 (16%) as medium risk and 7 (23%) as high risk for malnutrition. Initial nutritional assessment on admission was completed only in 4 patients (9%). Nutritional action plan was maintained completely only in 8 patients (18 %). Reassessment of MUST score was sub optimal in 18(56%) cases. Dietician advice was sought only in 5(42%) patients of high and medium risk of malnutrition.

Conclusions

12 out of 45(27%) of patients were identified as medium and high risk for malnutrition, in spite of poor overall screening of nutritional status. We may infer that the actual incidence of malnutrition could be higher in this cohort of patients, if the assessment was done accurately. Ongoing monitoring and actions taken to improve nutritional status needs significant improvement to meet up with the standard. This reflects lack of awareness and the poor knowledge in screening and assessment of nutritional status among health care professionals. This may contribute to poor out come in Ortho-geriatric practice. Therefore it is prudent to raise awareness, and improve knowledge and skills on issues pertaining to nutrition, among healthcare professionals for better outcome.

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DEVELOPMENT OF A METHOD TO CHARACTERISE TIBIAL SHAPE: IMPLICATIONS FOR THE SUCCESS OF THE OXFORD UNICOMPARTMENTAL KNEE ARTHROPLASTY

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The Oxford Unicompartmental knee arthroplasty (UKA) is indicated in patients with anteromedial osteoarthritis. Although the Oxford UKA preserves knee kinematics and function more than total knee replacement designs, outcome is variable with comparatively high revision rates reported. Proximal tibial (PT) shape shows considerable natural variation; however, it is unknown whether PT morphology has any impact on the functional outcome of the Oxford UKA.

AP radiographs from 78 patients with 81 medial Oxford UKA were analysed to characterise PT shape according to 7 parameters, 4 dimensional and 3 angular. These were: Total Tibial Area (TTA); Tibial Area Beneath Tibial Tray (TABT); Tibial Width (TW); Tibial Canal Flare Index (TCFI); Lateral Tibial Angle (LTA); Medial Shaft Angle (MSA); Lateral Shaft Angle (LSA). The aims of the study were threefold: (1) to assess the feasibility of using a semi-automated measurement process by comparing Active Shape Modelling (ASM) results to manual measurements, (2) to assess the validity of the characterisation by comparing the resulting distributions to known variation in PT shape, (3) to assess the impact of established variation in PT morphology on functional outcome using the Oxford Knee Score (OKS).

Results showed a high degree of correlation between ASM and manual measurements, particularly for the area measurements (TTA: 0.908, TABT: 0.922). Angular measurements were more reliably achieved using ASM (Inter-observer ICC LTA: 0.050). Bland-Altman plots demonstrated a consistent deviation in ASM dimensional results suggesting modification of the process is required (TTA MD: 330.37, TABT MD: 99.28). The validity of the characterisation was supported by trends in parameter variation according to gender, height, weight and age.

No association was found between PT shape and functional Oxford UKA outcome. However, this preliminary work was limited by cohort size and should be extended to allow for more high-powered analysis.

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ACCELERATED DEATH OF IN SITU CHONDROCYTES IN DRYING CARTILAGE BY LAMINAR AIRFLOW

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Cartilage drying results in chondrocyte death, the severity of which correlates with duration of exposure and varies throughout tissue depth. Additionally, intermittent irrigation has been shown to reduce, although not entirely eradicate, cell death[1]. This study has investigated the effect that airflow had on the trauma associated with cartilage drying by assessing the gross appearance of cartilage and the percentage of chondrocyte death.

Bovine metatarsophalangeal joints were dissected and exposed for a fixed time to either: 1) forced airflow (between 0-0.45m/s); 2) forced airflow (0.7m/s); 3) static air; 4) forced airflow (0.7m/s) covered by a saline soaked dressing; or 5) immersed in 0.9% saline solution. Joints from groups 2-5 were then immersed in 0.9% saline for 2hrs. Photographs recording gross changes were taken throughout and osteochondral explants harvested for live/dead fluorescent assay using confocal laser scanning microscopy. Data were obtained from at least n=5 independent experiments, p-values less than/equal to 0.05 were considered significant.

Cartilage immersed in saline or covered with a wet dressing retained its fresh appearance throughout. However in static air, cartilage changed colour and texture, particularly around raised features. These changes were more advanced and across the entire joint surface for cartilage exposed to forced airflow. Macroscopic changes were almost entirely reversed by joint immersion in 0.9% saline for 2hrs, meaning that the cartilage appeared relatively normal.

Cell death was observed to increase significantly with increasing airflow rate ($P<0.05$). Furthermore, all chondrocytes within cartilage dried under forced airflow (0.7m/s) were dead after 90mins (99.98+/-

0.02%). This was significantly (P<0.05) higher than both those dried in static air (3.17+/-2.0%) and under forced airflow but covered by wet a dressing (0.53+/-0.16%).
Re-hydration restores normal cartilage appearance, even in the absence of viable cells. Additionally, an increase in airflow rate exacerbates cell death resulting from drying. However, this can be prevented if exposed cartilage is covered with a saline soaked dressing. Chondrocyte death can lead to cartilage degeneration and therefore every effort should be made to prevent cartilage drying, particularly when laminar airflow is present.
1. Pun SY, Teng MS, & Kim HT. (2006). J Bone Joint Surg Br. 88:1528-1532.

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ASSESSMENT OF CHRONIC PAIN AFTER TOTAL KNEE ARTHROPLASTY:
DEVELOPMENT OF A CORE OUTCOME SET

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Background and objectives
Approximately 20% of patients experience chronic pain after total knee arthroplasty (TKA). The Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) includes pain in its recommendations for core outcomes to assess in clinical trials of pain treatment. However, pain is multidimensional and there are no recommendations about which aspects of pain should be assessed. In this project, we aim to identify measures used to assess chronic pain after TKA and develop a core outcome set for the assessment of chronic pain after TKA.
Methods
This ongoing project is funded through a NIHR Programme Development Grant on the treatment and management of chronic pain after TKA (the STAR programme). A systematic review was conducted to identify pain domains that are assessed after TKA. MEDLINE, Embase, PsycINFO, Cochrane Library and CINAHL databases were searched for research articles published in all languages between January 2002 and November 2011. Articles were eligible for inclusion if they assessed pain at a minimum of 3-months after TKR.
Results
Searches identified 1,164 articles which assessed pain at a minimum of 3-months after TKA. Fifty-four different multi-item tools containing pain questions were used in studies of TKA, with the American Knee Society Score most commonly used (used in 58% of studies). Some recent reduction in the use of clinically-administered tools was accompanied by increase in use of patient-reported outcome measures. The pain tools used were predominantly orientated towards assessing pain severity, and there was little assessment of other key aspects of pain, such as temporarily and quality.
Conclusions
Our systematic review identified wide variation in methods of pain assessment after TKA. Standardisation and improvements in assessment is needed to facilitate comparisons of results across studies and the identification and treatment of patients. We are currently developing a long list of pain domains using the results of this systematic review, interviews with patients and focus groups with healthcare professionals. We will then use a Delphi survey to develop a core outcome set for chronic pain after TKA. The results of the first round of the Delphi survey will be analysed and presented.

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USE OF HUMAN BONE IN A NOVEL THREE-DIMENSIONAL COCULTURE MODEL TO STUDY BREAST CANCER CELL GROWTH IN BONE

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Background and Objectives
Bone is a preferred site for secondary tumour growth in breast cancer. Currently, studying the interactions between bone and cancer cells in vitro relies on either monolayer co-cultures or cells grown on scaffolds in a specialised bioreactor. We have developed a model allowing us to culture human bone cores in vitro, providing not only the 3D environment but also the numerous cell types that encompass the human bone metastasis niche. We will use this novel model system to study cancer-bone cell interactions and effects of bone-targeted therapies.
Methods
Trabecular bone cores (5mm x 5mm) were isolated from the femoral heads of patients undergoing hip replacement surgery and cultured in DMEM (10% FBS, 1% penstrep and 1% fungizone) for 5 days on a moving platform with media changed every 48 hours. Bone cores were seeded with 1x104 DiD labeled MDA-MB-231-GFP breast cancer cells suspended in media or 30% matrigel (8 cores each). Bone architecture was assessed by microCT analysis before processing for histological analysis of bone cells. The presence of DiD-labeled MDA-MB-231 cells in snap frozen bone cores was investigated by multiphoton microscopy. Osteoclast activity was measured by CTX ELISA of the culture media.
Results
The use of matrigel to enhance adhesion of MDA-MB-231-GFP cells to bone cores provided no additional advantage compared to seeding in media alone. There was no change in bone volume, trabecular number or thickness of MDA-MB-231-GFP seeded bone cores compared to controls or baseline bone cores. A range of 0.11-0.71ng/ml of CTX was measured in media samples although there was no significant difference between any of the treatment groups. The presence of DiD-labeled MDA-MB-231 on the bone surfaces was confirmed by multiphoton microscopy following 9 days co-culture.
Conclusion
Human bone cores contained functional osteoclasts following 2 weeks of ex-vivo culture, and preservation of bone volume and integrity suggested concomitant bone formation. We demonstrated that MDA-MB-231 breast cancer cells colonises human bone cores in vitro. Future studies will use this co-culture system to generate new insight into the relationship between breast cancer cells and the human bone microenvironment.

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THE ROLE OF OSTEOARTHRITIC CYTOKINES IN A BONE MODEL OF ALKAPTONURIC ARTHROPATHY

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Background: Alkaptonuria (AKU) is a rare, hereditary disorder of autosomal recessive inheritance, caused by absence of homogentisate 1,2 dioxygenase, the enzyme responsible for the breakdown of homogentisic acid (HGA); a tyrosine degradation intermediate. Despite efficient renal excretion of HGA, levels remain elevated throughout body tissues and fluids. Over time HGA polymerises and is deposited within connective tissue, causing ochronosis (darkening of collagenous tissues). Long term ochronosis in articular tissues results in the development of ochronotic osteoarthropathy, often misdiagnosed as early onset osteoarthritis (OA).
Research into AKU has demonstrated that focal change within cartilage is required before the development of ochronotic osteoarthropathy, leading to speculation that there may be an overlap between the pathogenesis of OA and ochronotic osteoarthropathy. This study investigates the pathogenesis of the arthritis of AKU using an in vitro model, and assesses the effect of interleukins (ILs) involved in OA on this model.
Methods: Osteosarcoma cell line (MG63) was cultured for 10 and 21 days in medium containing either 0.33M HGA or 1ng/ml IL-1, 6 and 10 or a combination of HGA and ILs. Cultures were stained with Schmorls reagent and nuclear fast red for histological examination to quantify pigmentation, and the effect of reagents on cell viability was analysed by trypan blue assay.

Results: Cell cultures treated with HGA (solely or in combination with ILs) demonstrated significantly reduced cell viability and clear intra- and extracellular pigment deposition compared to control and IL cultures.
Statistical analysis of pigment deposition demonstrated that cells cultured for 10 days in HGA + IL-1, and 21 days culture in HGA + IL-6 and HGA + IL-10 had a significant difference ($p<0.001$, $p<0.01$ and $p<0.001$ respectively) compared with any other groups.
Conclusions: These results demonstrate that ILs combined with HGA increase pigment deposition. It appears that pigmentation observed in cultures treated with HGA, alone or in combination with ILs, has a greater effect on cell viability than IL solely. This suggests that the arthropathy of AKU is more severe and detrimental to the bone cells of the joint compared to OA, possibly explaining why arthropathy in AKU progresses more rapidly than OA.

P 69

GENETIC LACTOSE TOLERANCE AND REDUCED RISK OF HIP FRACTURE - A MENDELIAN RANDOMIZATION STUDY.

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The genetic variant C/T-13910 in the MCM6 gene associates with lactose intolerance/tolerance. The genotypes TT and TC are associated with lifelong lactose tolerance, whereas CC associates with adult lactose intolerance. People with lactose intolerance consume less milk compared to tolerant people. Meta-analyses of randomised trials and prospective cohort studies have shown that D-vitamin supplements may prevent hip-fracture, but calcium supplements or milk intake do not show any evidence of a preventive effect on risk of hip fracture. Furthermore, small-scale genetic studies indicate a modest risk of hip fracture in elderly women carrying CC, but the effect in men and younger women is unsure. We investigate whether elevated milk intake is causally associated with reduced risk of hip fracture using the genetic variant as a measure of lifelong milk-exposure
We included 93,197 people aged 30-99 from 3 Danish population studies (the Copenhagen City Heart study (CCHS); the Copenhagen General Population Study (CGPS) & the General Suburban Population Study (GESUS)). All participants were genotyped. Milk intake was self-reported in GESUS and CGPS. Endpoints were obtained from Danish registries. 9 years follow-up period was conducted on the first hip fracture on 72,905 participants from CGPS. We used a Mendelian Randomization design and instrumental variable analysis was used to obtain a causal estimate
Intake of milk was lowest among people with genotype CC (mean glasses/week: CC=5, TC=7, TT=7; P=0.0001). The total number of hip fractures was 1,764. No significant associations were found between milk intake or genotypes and hip fracture using incidence or prevalence data respectively. For hip fracture the observationally hazard ratio for an increase in milk intake of 1 glass of milk/week was 1.00(0.99-1.01). The corresponding causal odds ratio for hip fracture was 1.02(0.91-1.16).
No causal inference can be draw from this study between elevated milk intake and reduced risk of hip fracture using the genetic variant of LCT-13910 C/T. Therefore, increased milk intake does not associate with reduced risk of hip fracture, observationally or causally.

P 70

ENRICHMENT OF SKELETAL STEM CELLS USING STRO-1 AND PERIVASCULAR MARKERS CD146 AND CD105 (ENDOGLIN) INDIVIDUALLY AND IN COMBINATION - A COMPARATIVE STUDY

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INTRODUCTION: Skeletal stem cells (SSCs) provide an ideal cell source for bone tissue engineering strategies to meet the medical challenges presented by diseases such as osteoporosis, prevalent within an increasingly aged population. However, the isolation of homogeneous SSC populations from human bone marrow (HBM) remains a research objective. Stro-1 is a robust and reliable SSC surface marker; however, Stro-1+ populations exhibit variable colony forming unit-fibroblastic (CFU-F) capacity and osteogenic differentiation potential (bone stem cell pre-requisites). SSCs are thought to originate from the sub-endothelial or perivascular niche residing there as adventitial reticular cells. The present study has investigated the use of the pericyte marker CD146 and endothelial marker CD105 as alternative or additional markers to Stro-1 for SSC enrichment.
METHODS: Marker expression was quantified by flow cytometry within osteoarthritic HBM before magnetic-activated cell sorting of single immuno-labelled populations and fluorescence-activated cell sorting of dual immuno-labelled populations. Isolated populations were then characterised in vitro for CFE capacity, alkaline phosphatase (ALP) activity (an indicator of osteoblast differentiation) and osteogenic gene expression (ALP, CADM1, CLEC3B, DCN, LOXL4, OPN, POSTN and SATB2). In vivo characterisation consisted of subcutaneous implantation in MF1-nu mice within diffusion chambers.
RESULTS: CD146+ and Stro-1+ populations exhibited both enriched CFE capacity as determined by percentage ALP+ colony formation, and new collagen/proteoglycan deposition in vivo following subcutaneous implantation within the peritoneal cavity of immunodeficient mice, compared to control (unlabelled HBM) and CD105+ populations. Interestingly, gene expression analysis of select osteogenic genes showed Stro-1+ and CD146+ populations were not significantly different. Indeed, a small HBM stromal cell (HBMSC) fraction (2.04% +/- 0.41%) immuno-labelled for Stro-1+/CD146+.
DISCUSSION & CONCLUSIONS: The data indicate CD146 as a potential additional SSC enrichment marker to Stro-1, targeting a narrower HBMSC population. However, molecular analysis demonstrated no significant difference between these enriched skeletal populations for the genes investigated. The current studies highlight a further requirement for novel SSC specific markers to isolate populations for implementation in tissue engineering and regenerative medicine strategies.

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IDENTIFICATION OF MOLECULAR DETERMINANTS OF MYELOMA CELL ENGAGEMENT IN THE OSTEOBLASTIC NICHE IN BONE

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Introduction:
Multiple myeloma is an incurable haematological malignancy, caused by the expansion of malignant plasma cells within the bone marrow (BM). It has been suggested that chemo-resistant myeloma cells reside within a protective osteoblastic niche, similar to that occupied by quiescent haemopoietic stem cells (HSCs), resulting in myeloma cell survival via chemotactic and adhesion molecules.
We hypothesise that myeloma cells express molecules also expressed by HSCs (CXCR4, Notch-1, Tie-2 and N-cadherin) which interact with their ligands (CXCL12, Jagged-1, Angiopoietin-1 and N-cadherin) to promote myeloma cell dissemination to an osteoblastic niche. Disruption of anchoring molecules will inhibit myeloma cell adhesion to osteoblastic cells which in turn will result in chemo-sensitisation.
Methods:
In vitro expression of molecules expressed by HSCs and complimentary ligands by murine 5TGM1MMvt-GFP, MC3T3-E1 and primary osteoblastic cells was determined using RT-PCR, FACS and immunofluorescence.
5TGM1MMvt-GFP cells were injected into C57BLKaLwRij mice and at 10, 14, 17 and 21 days post-injection, ex vivo expression of each molecule was determined by the BM using FACS.

5x104 control and N-cadherin knock-down 5TGM1MMvt-GFP cells (generated using lenti-viral shRNA technology) were seeded onto differentiated primary osteoblasts and adhesion was measured using fluorescent microscopy after 1 and 6 hours.
Results:
CXCR4, Notch-1, Tie-2 and N-cadherin were expressed by 5TGM1MMvt-GFP cells in vitro, and ex vivo expression of each molecule was significantly reduced (excluding CXCR4) 21 days post-injection, compared to in vitro cells.
CXCL12, Jagged-1, Angiopoietin-1 and N-cadherin were expressed by MC3T3-E1 and primary osteoblastic cells in vitro.
Knock-down of one of these molecules, N-cadherin, in the 5TGM1MMvt-GFP cells did not significantly inhibit adhesion to osteoblasts in vitro compared to control 5TGM1MMvt-GFP cells, suggesting that other molecules may be more important in this interaction.
Conclusions:
The expression of HSC molecules and ligands by myeloma cells and osteoblasts supports the hypothesis that myeloma cells engage in the same niche to that occupied by HSCs. However, the role of each molecule is yet to be determined. The knock-down of N-cadherin in 5TGM1MMvt-GFP cells did not significantly impair adhesion to osteoblasts in vitro however; may suggest that one molecule is not solely responsible for anchoring myeloma cells to osteoblasts.

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SIMULATION OF MECHANICALLY ADAPTIVE BONE REMODELLING IN THE PROXIMAL FEMUR USING TOPOLOGY OPTIMIZATION

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Structural efficiency is very important in the muscular-skeletal system. There is a clear evolutionary advantage in a skeleton that has suitable stiffness and strength to support all applied loads without failure, while keeping bone mass as low as possible to minimize the weight and the metabolic cost of maintaining the bone tissue. The external geometry of a bone is dictated by the requirements of its function, e.g. joint location and muscle attachment. However, observational studies of the internal trabecular bone structure have shown that the arrangement of this structure is actively influenced by the mechanical loads applied to the bone. The objective of this mechanically adaptive bone remodelling process, high stiffness and low mass, is analogous to the common objective of topology optimization. This suggests that cancellous bone is a self-optimizing structure and that the structure produced by mechanically adaptive bone remodelling could be predicted using topology optimization.

Topology optimization is an engineering technique used to determine the best arrangement of material within a structure. The common objective of this process is to minimize the structural compliance while fulfilling a maximum material volume constraint. In this investigation the level set method of topology optimization is used to optimize the internal structure of a 3D model of the proximal femur. It is hypothesized that the internal bone architecture would be a mechanically optimal structure if the trabecular arrangements observed within the real femurs are similar to the optimal structure predicted by topology optimization. Topology optimization cannot account for the bone structure's metabolic requirements; so differences between the real and modelled bone structures could reveal the mechanical and metabolic features of the trabecular bone architecture.

If the internal trabecular bone structure can be shown to be a self-optimizing structure then topology optimization would provide a robust and efficient method for predicting the effect of changes in bone loading on the internal bone structure. Topology optimization could be used as a design tool for orthopaedic implants, identifying design features that preserve bone stock and reduce stress shielding.

P 73

THE EFFECT OF IMPLEMENTING ELECTRONIC OPERATION NOTES IN TRAUMA AND ORTHOPAEDICS

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Background & Objectives
Accurate and detailed documentation of surgical operation notes is crucial, both for post-operative management of patients and for medico-legal clarity.
The aims of this study were to compare operation documentation against the Royal College of Surgeons of England (RCS) guidelines and to compare the before-and-after effect of introducing an electronic operation note system.
Methods
A retrospective audit of operation notes was carried out at a London Major Trauma Centre, with the audit standard set by the RCS guidelines. In December 2012, 50 consecutive operation notes for inpatients that had undergone emergency orthopaedic trauma surgery were audited.
The findings were presented and in addition to educating surgeons, an electronic operation note proforma was then introduced and a re-audit carried out after its implementation.
Results
98% documented the date of the surgery but none included either the time of surgery or age of the patient. 84% stated the incision but only 60% included the closure details. Merely 69% included the antibiotics given at induction. Crucially, only 66% of the operation notes had legible hand writing.
The results after implementation of electronic operation notes, demonstrated a marked improvement. All notes contained an operation note (previously 5/6). 75% included time of surgery and age of patient and 100% included the date. 100% included closure details, antibiotic selection at induction, signature and post-operative instructions. All were typed, making for 100% legibility.
Conclusion
We used our pilot audit to target specific information that was commonly omitted and we 'enforced' these areas using drop-down selections in electronic operation note.
The re-audit after its implementation showed a clear improvement in documentation. Importantly, the legibility of notes (previously 66%) was ensured by typing and printing in theatre. All were saved on a secured network database and therefore could easily be accessed by surgeons and staff if patient notes were missing or unavailable.
This study has demonstrated that implementation of an electronic operation note system markedly improved the quality of documentation, both in terms of information detail and readability. We would recommend this template system as a standard for operation note documentation.

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NON SURGICAL MANAGEMENT OF AN AVULSION FRACTURE INJURY OF EXTENSOR CARPI RADIALIS BREVIS

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Avulsion fractures at the base of the index or middle finger metacarpals are rare with no consensus regarding the optimal management of these injuries. We describe an avulsion fracture injury of extensor carpi radialis brevis (ECRB) insertion treated non-surgically, which has been previously undescribed.
A 63 year old woman presented with a tender, hard lump over the third metacarpal base ten days post wrist injury. She was treated for six weeks in a volar splint. The pain settled within six weeks: at one year she had regained full range of movement and strength.
The relative stable bone and soft tissue architecture of the radial carpo-metacarpal joints (CMCJ), means fractures and dislocations in this region are less common. This prevents dorsal dislocation of the third CMCJ during a forced hyper-flexion injury at the wrist: an avulsion injury is therefore seen, rather than a dislocation. These injuries can be difficult to assess and in this case dorsal fragment was only visible on the lateral radiograph.
Surgical intervention can restore joint surface integrity and prevent formation of metacarpal boss. There are no reported cases of non-surgical treatment of an ECRB avulsion fracture. We have satisfactorily treated our patient without surgery. Joint congruity at the third CMCJ may not be an absolute priority owing to the limited movement at this

joint. Furthermore, conservative management avoids risks associated with surgery.
Avulsion fractures of the ECRB are rare and require careful clinical and radiographic assessment for diagnosis. Non-surgical management of this patient achieved a satisfactory result.

P 75

ISOLATED PROXIMAL RUPTURE OF FLEXOR DIGITORUM LONGUS TENDON IN A TRAUMATIC OPEN SUB-TALAR DISLOCATION: A CASE REPORT

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We report a case of an open subtalar dislocation and associated isolated proximal rupture of flexor digitorum longus (FDL) tendon at the musculo-tendinous junction, following a relatively low energy trauma.
Tendon ruptures associated with ankle fractures/dislocations or subtalar dislocations are very rare entities and there are only a few reports of these in the literature. There has previously been described a case of simultaneous rupture of the tibialis posterior and FDL tendon, associated with a closed distal tibial fracture. To the Authors' knowledge, however, no previous cases of isolated rupture of FDL associated with an open subtalar dislocation have been described.
The finding of the avulsed FDL tendon was an intra-operative one and was managed by a primary side-to-side tenodesis with flexor hallucis longus tendon. The patient has made a good functional recovery from his injuries.
This case is reported because of the rarity of this combination of injuries and the associated management dilemma it presented us.

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COMPARISON OF EXETER AND ULTIMA-TPS FEMORAL STEMS USING NOVEL, REGION-FREE DUAL ENERGY X-RAY ABSORPTIOMETRY ANALYSIS SOFTWARE (DXA-RFA)

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The Exeter (Howmedica Ltd) and Ultima-TPS (Depuy Ltd) implants are both collarless, polished, double-tapered, cemented femoral implants. The Exeter is manufactured in stainless steel whilst the Ultima-TPS is manufactured in cobalt-chrome. A previous short term follow-up dual energy X-ray absorptiometry (DXA) study found no difference in bone mineral density (BMD) change between the 2 implants, however it was performed using traditional region of interest (ROI) analysis methods. The recent development of region-free DXA analysis software (DXA-RFA) now allows BMD measurement on a pixel-by-pixel basis from a DXA scan, allowing far more detailed comparison of the 2 prostheses and the identification of the exact areas where greatest BMD change occurs following total hip arthroplasty (THA).
Twenty-five patients with unilateral hip osteoarthritis were recruited as part of a previous study and randomised to receive either the Exeter or TPS stem, with all patients receiving a Charnley cup. Bone mineral density was measured post-operatively and at 3 and 12 months follow-up. Eleven patients (mean age 71 years, 10 female, BMI 28.6Kg/metres squared) received the TPS implant and fourteen patients (mean age 72 years, 12 female, BMI 28.1kg/metres squared) the Exeter implant.
A common master template was generated that was the average shape of all of the proximal femur images within the study and each femur was mapped to the template, ensuring the consistent positioning of each pixel of a scan. Images were produced showing average BMD change from baseline at each pixel for the two groups of patients and images were generated to show any areas of different BMD change between the two implants. P-value pixel maps were also produced that allowed the identification of any pixels that showed significant BMD change during the follow-up period.
This study suggests that the early pattern of bone change in the proximal femur is similar between the two implants studied, although the pattern of bone gain in the greater trochanter varied considerably between the two implants. The study also shows how insensitive the commonly used 7 ROI model developed by Gruen is at identifying the precise locations where greatest BMD change occurs following THA.

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JOINT SHAPE AS A PREDICTOR OF END-STAGE OSTEOARTHRITIS OF THE HIP

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Abnormalities in hip shape are thought to be important factors in the development of osteoarthritis (OA) in the hip joint. In this study Statistical Shape Modelling (SSM) was used to examine the relationship between hip shape and risk of end-stage OA in a group of women from the Chingford cohort who have undergone total hip arthroplasty (THA). The results were compared with a previous study by Nicholls et al. (Arthritis Rheum. 2011) where morphological parameters of the hip were measured in the same subjects (n=135) using Hip Morf software.
An 83 point SSM template was applied to each hip joint from pelvic radiographs taken at year 2. It included the proximal femur, osteophytes and part of the pelvis. To ensure equal influence of OA and control subjects in the SSM, 44 images were used to build the model: 22 who had undergone THA by year 20 (THA group) and 22 randomly selected controls who were THA-free in both hips at year 20 (control group). The model was then used to analyse all 268 hips selected for Hip Morf analysis, 25 THA and 242 controls (One with an indistinct outline was excluded). The first 15 scores of shape variance, or mode scores, were calculated for each hip.
Mode 5 was significantly lower in the THA group compared with the control group, (P=0.039). A lower Mode 5 score was significantly correlated (Pearson) with 8 of the 10 morphological parameters from the Hip Morf study which were significantly associated (P<0.05) with an increased risk of THA. These included two deformities commonly associated with hip OA: a) acetabular deformity, with a lower lateral centre edge angle and higher extrusion index and b) cam or 'pistol grip' deformity (shown in figure) with a higher alpha angle and increased modified triangular index height. Mode 5 remained significant after adjustment for age, BMI and Kellgren Lawrence Grade, (P=0.026), odds ratio 0.54 (95% CI 0.31-0.93).
This study showed that SSM can be used to quantify the changes in shape of the hip joint and may help to identify subjects at highest risk of requiring a THA.

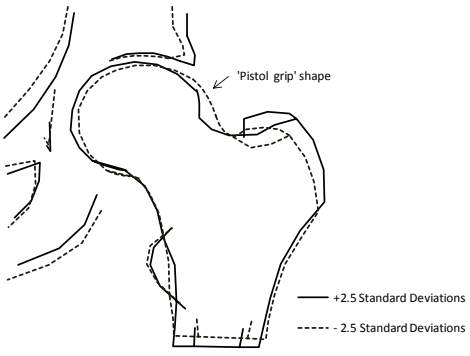

Figure showing shape variation associated with Mode 5. Low scores (dashed outline) are significantly associated with an increased risk of THA at 20 years compared to high scores (solid line).

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MODULATING WNT SIGNALLING IN SKELETAL STEM CELLS FOR BONE REGENERATION

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Bone-related illnesses are among major public health problems, with osteoporosis treatment costing the UK £2 billion each year. Furthermore, 10% of all bone fractures fail to heal properly, resulting in non-unions. We hypothesise that manipulating the growth and differentiation capacity of stem cell populations within the bone (called skeletal stem cells, SSCs) may help address these problems. As the Wnt signalling pathway has been shown to be involved in regulating SSCs, we tested the influence of Wnt induction in stem cells at different stages of osteogenic commitment for future therapeutic strategies involving targeted delivery of Wnts.

We investigated the effects of different concentrations (25-100ng/ml) and time exposures (24h or 14 days) of Wnt3a protein added to basal and osteogenic media of freshly isolated human bone marrow cell populations (mixed bone marrow mononuclear cell populations, BMMNCs, and STRO-1-selected osteoprogenitor-enriched populations). Progenitor/osteogenic commitment were measured by FACS analysis for STRO-1, alkaline phosphatase (ALP) and other SSC markers as well as by colony forming assays (CFU-F and CFU-O) and ALP staining. ALP activity and expression of osteogenic genes was also assessed.

Expression of STRO-1, a marker of osteoprogenitors, was increased significantly in BMMNCs after 24h of exposure to 100ng/ml Wnt. Culture of BMMNCs under basal or osteogenic conditions for 14 days after an initial 24h exposure to 100ng/ml Wnt3a immediately after isolation resulted in an increase in subsequent osteogenic differentiation, measured by a 23.15%+/-9.95% increase in ALP staining. However, long-term activation of the Wnt pathway had an opposite effect, reducing osteogenic differentiation, measured by an average 12x decrease in ALP activity. We are now conducting preliminary experiments to test the possibility of Wnt delivery via liposomes, and its activity within these nanoparticles, targeted to desired stem cell populations of the bone marrow via STRO-1 antibody. This is to ensure greater osteogenic specificity of our approach.

Our results indicate that Wnt signalling has strikingly different effects on osteogenic differentiation in bone marrow-derived stromal cells, depending on their osteogenic commitment. Selective targeting of Wnt proteins or agonists to specific cell populations therefore might be a safe and efficacious therapeutic approach for promoting bone regeneration.

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IMMOBILIZED VEGF ON TITANIUM SCREWS TO PROMOTE IMPLANT INTEGRATION AND VASCULAR REGENERATION

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Biomedical engineering has the potential to develop prosthetic substitutes that may alleviate musculoskeletal disease conditions and help improve the quality of living. Prime examples of such implants include joint replacement components and fracture fixation devices. A number of clinical problems are still associated with their use, such as lack of host tissue integration and implant failure related to poor bone healing adjacent to the implant. Successful implant integration into the surrounding tissue is highly dependent on the crucial role of blood supply in driving bone repair and development. Immobilization of growth factors onto the surface of metal substrates and biomaterials may be a viable approach in promoting revascularization and enhanced implant integration in a controllable manner. VEGF or vascular endothelial growth factor and angiopoietins (Ang-1 and Ang-2) bioactive factors can promote revascularization on in vitro models. We investigated the effects of immobilized VEGF on titanium alloy screws coated with thin adherent polydopamine film on a rabbit model.

Titanium screws were coated with or without polydopamine or VEGF. Titanium screws of 3.5 mm x 10 mm size were implanted in the cancellous bone at the lateral epicondyle of femur. In the cortical bone on the metaphysis, 2 cm below the tibia tuberosity were implanted 2 screws of 2.7 mm x 8 mm dimensions. Time points after implantations were 6 weeks and 12 weeks. Gross X-ray imaging, immunostaining and pull-out test were used to evaluate the integration of the titanium screws.

The pull-out test of the polydopamine coating improved implant integration into the host bone compared to pristine titanium screws at 6 weeks relative to 12 weeks. Histological results suggest improved implant integration compared to controls. These results suggest that VEGF can be used to improve implant integration at earlier time point of 6 weeks.

Polydopamine has the benefit of improving titanium screw integration into bone compared to pristine titanium. VEGF-coated titanium screws have the benefit of improving early integration compared to controls.

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IS OSTEOCLAST ACTIVITY AFFECTED BY ADENOVIRUS INFECTION?

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Osteoclast resorption depends on their ability to reorganise their actin cytoskeleton and form the sealing zone. In order to resorb bone, osteoclasts become polarised by condensing their podosomes into a highly dynamic podosomal belt. The podosome turnover is regulated by several factors such as non-receptor tyrosine kinases, small GTPases and actin-binding proteins. The innate immune system responds to viral pathogens. Cytoplasmic double-stranded DNA activates the immune system inducing interferon (IFN) production, inflammasome activation, and cell death. We studied whether transfecting osteoclasts with DNA affected their differentiation and resorption ability. The differentiation and activity of adenovirus infected human osteoclasts was determined relative to non-infected cells. By analysing the formation of tartrate-resistant acid phosphatase (TRAP) positive cells, no effect on osteoclasts differentiation was observed however, a reduction in resorption was found. Early infection significantly inhibited osteoclasts resorption compared to late infection. MTT cell viability assay determined no effect on osteoclast cell viability following transfection. Interestingly, an increase in tumor necrosis factor stimulated gene-6 (TSG-6) expression was observed in infected osteoclasts. TSG-6 expression is known to be induced in response to inflammatory cytokines and to downregulate osteoclasts activity.

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COMPUTER-ASSISTED METHOD FOR THE DIRECT SPATIAL 3D MAPPING OF TRABECULAR DISCONNECTIONS IN THE SPINE: A POTENTIAL TOOL IN THE SURGICAL PROPHYLAXIS OF VERTEBRAL FRACTURES?

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Bone loss with age makes the skeleton fracture-prone in the rapidly expanding elderly population. 300,000 new fragility fractures occur annually in the UK, 120,000+ of these being vertebral compression fractures (VCF). Some VCFs cause life-altering pain, requiring surgical intervention. Vertebroplasty is a minimally invasive procedure whereby bone cement is injected into the damaged vertebral body. However, vertebroplasty can alter the biomechanics, apparently leaving adjacent vertebrae with an increased VCF risk. Prophylactic augmentation of intact, though at-risk, vertebrae may reduce the risk of adverse effects. The question therefore arises as to which areas of a non-fractured vertebral body structurally weakened with age, and thus should be targeted. Frequent reports of an overlap in BMD between fracture and non-fracture subjects suggest the combination of bone quantity and quality (microarchitectural strength) may be a more reliable fracture predictor than BMD alone. Traditional histological methods for microarchitectural interconnection (a significant bone strength factor) are limited as they usually indirectly extrapolate 3D structure from thin 2D sections. Aaron et al (2000) developed a novel, thick slicing and superficial staining procedure, whereby unstained real (not stained planar artifactual) trabecular termini (ReTm) are identified directly within their 3D context. The aim of this study was to automate a method of identifying trabecular regions of weakness in vertebral

bodies from ageing spines. Embalmed cadaveric vertebral bodies were scanned by MicroCT, before plastic-embedding, slicing thickly, and surface-staining. The ReTm were mapped using light microscopy to demonstrate any apparent loci of structural disconnectivity that may cause weakness. A transparent 3D envelope corresponding to the cortex, was constructed using code developed in-house (Matlab), and validated by overlay of the previous MicroCT scan and the coordinate data. The ReTm distribution was remarkably heterogeneous ($p<0.05$) and independent of the bone volume ($p<0.05$). There was preliminary evidence of central endplate disconnection predominantly. Such automated spatial mapping of the ReTm within a 3D framework overcomes the constraints of 2D histology. Patterns of trabecular disconnection in the spine may provide insight into specific regional atrophy, perhaps explaining why some vertebrae fracture while others with the same BMD do not, and indicating better targets for prophylactic vertebroplasty.

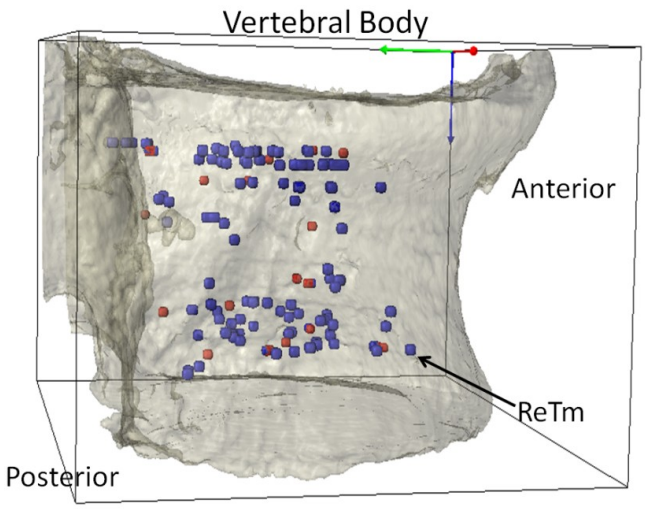

Figure 1. Computer generated construct of a human vertebral body showing the spatial mapping of
structural weakness in the form of 'real' trabecular termini (ReTm).

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QUANTIFICATION OF CELL NETWORKS: COMPUTER-ASSISTED METHOD FOR 3D ASSESSMENT AND COMPARISON OF OSTEOCYTE SYNCITIA IN THE AGEING HUMAN FEMUR

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Osteocytes are the most abundant bone cell (90-95%) and in normal tissue each may remain viable for many years. Despite entombment in the calcified matrix their extensive processes form a pervasive syncitium, similar to that of neurones in the central nervous system, and whereby no part of bone is more than a few microns from a cell. A role in mechanotransduction has been proposed for the network whereby it directs remodelling and repair. To assess regional variability and the morphological influence of stress input, a novel method has been developed that combines undecalcified histology, confocal microscopy (CLSM) and image analysis software to enable reliable and convenient 3D quantitative characterisation of these osteocyte syncitia, with special reference to the cancellous bone at the hip (a particularly vulnerable skeletal site). Surgically discarded human femoral heads, from ageing female patients, were used to compare the network in traditionally low stress (osteoporosis, OP, n=6, mean age of 82±13 years) and high stress (osteoarthritis, OA, n=7, mean age of 74±6 years) conditions. Segments, each containing a region of sub-foveal bone from the insertion region of the ligamentum teres, were en-bloc stained in calcein fluorochrome before embedding in resin. Slices, 300 microns thick, were examined by CLSM. Individual 2D Tiff images were imported into software (ScanIP, Simpleware, UK) that generated complementary 3D binary masks specifically representing cell body and process components along with the unwanted background component i.e. endosteum. Corresponding in-house code (Matlab 7.3, Mathworks, USA) was written to quantitate the complementary paired masked aspects including the number of cells, their length, and the volume and interconnection of processes. Method validation was undertaken utilising user-generated objects of known size and shape and reconstructed z-stacks of sequential images obtained from the CLSM in the form of AVI files to allow full visualisation. In OP, cells were less prevalent with fewer processes, forming a lesser interconnected syncitium than that found in OA, suggesting a reduced ability to communicate and orchestrate repair. This novel method apparently enables topographic appraisal of the syncitium, and the prospect of a more precise evaluation of its potential for biomechanical exchange in ageing and disease.

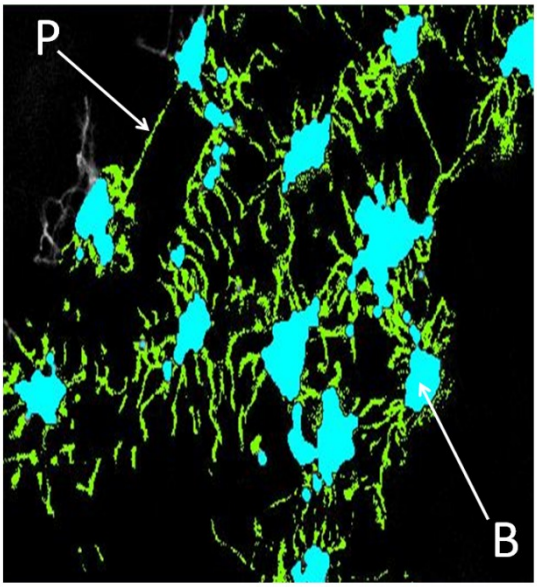

Figure 2. A 2D TIFF image of an osteocyte syncitium, from within osteoarthritic bone, showing the application of two binary masks for the quantification of the network, one representing the osteocyte cell body elements (B, blue), and the other the cytoplasmic processes (P, green).

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EPIDERMAL AND PLATELET DERIVED GROWTH FACTORS ELICIT WNT INDEPEDENT BETA-CATENIN SIGNALLING IN MESENCHYMAL STROMAL CELLS VIA INTEGRIN-LINKED KINASE

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Therapies targeting osteogenic commitment and differentiation of mesenchymal stromal cells (MSCs) to enhance bone growth and repair are urgently required and stimulators of the Wnt signalling pathway to act as bone anabolics are receiving considerable attention. On binding to its receptor complex, Wnt acts to inhibit GSK3-beta, which allows accumulation and nuclear translocation of beta-catenin, driving expression of Wnt-responsive genes. We have identified a Wnt ligand-independent mechanism for exerting control over beta-catenin through integrin-linked kinase (ILK), which acts downstream of receptor tyrosine kinases (RTK) in concert with integrin-activation, and can also target GSK3-beta for inhibition. From analyses of individual clones of immortalised human MSCs, we identified inter-clone variations in expression of the EGF/PDGF RTK signalling network, corresponding with elevated ILK and endogenously high active beta-catenin. Treatment with Dkk1, a receptor-level inhibitor of the Wnt pathway, had no effect on levels of active beta-catenin, measured by Western blotting using an antibody specific for the dephosphorylated form of the protein, pointing to a Wnt ligand-independent signalling event. When the MSC lines were exposed to specific inhibitors of EGF, PDGF and ILK, we observed a reduction in active beta-catenin. This provides evidence for a functional link between RTK signalling and beta-catenin activity via ILK in MSC sub-populations. To determine if similar signalling systems operated in primary cells, bone/marrow-derived MSCs were treated with recombinant EGF and PDGF in the presence and absence of an ILK inhibitor. Using western blotting we demonstrated that both EGF and PDGF induced increased active beta-

catenin expression at 24h, an effect blocked by ILK inhibition. Furthermore, ILK inhibition prolonged expression of pERK, an early mediator of EGF/PDGF signalling, suggesting a principal role for ILK in regulating short-term (pERK) and medium-term (beta-catenin) RTK responses in MSCs. Immunohistochemistry using an ILK-specific antibody identified discrete ILK-positive sub-populations of primary MSCs, which varied from 6-13% across donors, confirming MSC heterogeneity, which most likely disguised this previously unrecognised signalling pathway. Our evidence that the Wnt/beta-catenin signalling axis can be independently regulated by RTK growth factors in MSCs, and potentially integrin-mediated adhesive interactions, will have pervasive implications for bone biology and developing Wnt-based anabolics.

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A NEW METHOD FOR COMPARING ANKLE FRACTURE STABILISING CASTS

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Ankle fractures in elderly people are common and difficult to treat. A recent Cochrane review failed to differentiate between Standard Casting (SC) and Open Reduction Internal Fixation (ORIF) in adults (Donken et al 2012), but this result may not hold for older people with slower healing, fragile skin and higher risks of infection. New forms of casting are therefore under investigation, including Close Contact Casting (CCC), adapted from the total contact cast used to treat diabetic leg ulcers. The objectives of this study were to design a test method to assess the mechanical stability provided by different ankle casting methods, including a model of leg swelling reduction, and to compare SC and CCC methods.

Two identical adult mannequin legs were sectioned at the level of the malleoli and filled with resin, embedding a low-friction axial dowel connection between the foot and the shank. One leg additionally incorporated a section of bicycle inner tube representing oedema and its subsequent reduction with an increase of the minor axis of the ankle by 1/3. Casts were applied to these by a single surgeon experienced in both methods and the torque - rotation characteristics about the long axis of the shank were measured in a mechanical testing machine.

Ten repeats of torque - rotation data (Fig 1) were obtained for three SC and three CCC casts on each legs. Preliminary analysis at low rotation angles (+/- 20 degrees) showed CCC had higher mean stiffness than SC but CCC had a similar low stiffness range of angles to SC. On a swollen leg model, CCC showed higher mean stiffness but a greater loss of stiffness following reduction of ankle swelling.

This is the first ever mechanical test of ankle fracture casting stability. Torsion of the shank was chosen as a mechanical testing paradigm due to the vulnerability of ankle fractures to this loading and its likelihood of occurring during activities of daily living. These data confirm the mechanical similarity of CCC and SC. Further analysis will evaluate the statistical significance of this result and of the potential for CCC being more sensitive to swelling reduction than SC.

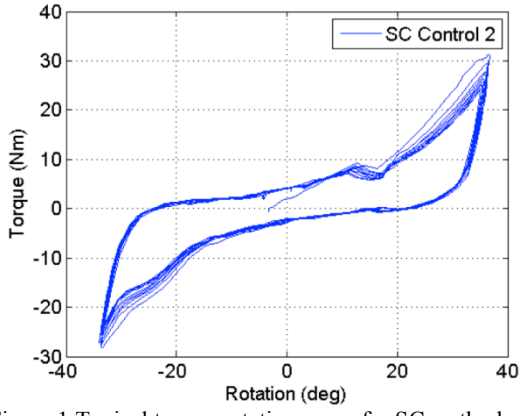

Figure 1 Typical torque rotation curve for SC method.

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LOCAL ANAESTHETIC AND CORTICOSTEROIDS ALTER MECHANICAL PROPERTIES OF ARTICULAR CARTILAGE

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Intra-articular injections of corticosteroids and anaesthetics are routinely to provide rapid short-term symptomatic relief, reduce inflammation, and increase mobility in patients with osteoarthritis and rheumatoid arthritis. However, injectable corticosteroids may have chondrotoxic effects, release destructive enzymes, and alter the affect the fluid and electrolyte balance of articular cartilage. The literature discussing the potential damaging effects of corticosteroids remains largely incomplete. Studies identifying cellular toxisity and gross tissue structural changes have not investigated specifically into how extracellular matrix alterations may alter the mechanical properties of articular cartilage. The aim of the proposed research is to compare how commonly used corticosteroids and local anaesthetic agents may affect the mechanical properties of articular cartilage.

1cm2 osteochondral specimens were obtained from the central load bearing area of porcine talar trochlea. Specimens were equilibrated for 24 hours in the suggested dosages of the following (n=8 per group): Control: 5ml Phosphate buffered saline with protease inhibitors (PBS+PI); Local anaesthetic: 0.50% (10ml) Marcain (bupivacaine); 1% (5ml) and 2% (5ml) Lidocaine Corticosteroid: 40mg Depo-medrone (methylprednisone acetate); 25mg/ml (1ml) Hydrocortisone Acetate. The articular cartilage surface was indented to 150 g at 0.5mm/second using a materials testing machine and both the Young's modulus and time-dependent stress behaviour analysed.

Young's modulus revealed no significant differences amongst the treatments and controls. However, when stress was plotted against time, samples exposed to either 0.5% Marcain (6.7 + 0.93 Mpa) or 40 mg Depo-medrone (5.5 + 2.1 Mpa), reached maximum stress levels over a shorter period of time than controls (3.26 + 1.2 Mpa). This indicates that exposure to these substances may alter the time-dependent properties of the tissue.

Although preliminary, the current investigation suggests that the time-dependent properties of articular cartilage may be altered through using Marcain and Depo-medrone. The potential for intra-articular injections of local anaesthetic and corticosteroids to alter the mechanical properties of cartilage may serve as a guide to the medical community about the potential deleterious affects of intra-articular local anaesthetic agent and corticosteroid injections on articular cartilage.

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MAXIMUM ALPHA ANGLE MEASURED ON MRI RADIAL SLICES HAS THE STRONGEST CORRELATION WITH EARLY HIP OSTEOARTHRITIS

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Longitudinal studies demonstrate that femoroacetabular impingement (FAI), defined as a raised alpha angle on anteroposterior (AP) radiographs, increases the risk of developing hip osteoarthritis. The commonest form of FAI arises when additional bone (cam lesion) at the anterosuperior femoral head-neck junction abuts the acetabular rim. This causes localised cartilage damage that can be detected using delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC). Our aim was to determine which imaging strategy for the assessment of cam lesion morphology best correlates with early degenerative change assessed using dGEMRIC.

31 asymptomatic individuals (mean age 52 years) from a longitudinal cohort study investigating hip osteoarthritis underwent standing AP and cross-table lateral radiographs, 3D morphological MRI, and dGEMRIC of their index hip at 3T. A dGEMRIC index was calculated as a ratio of

the anterosuperior acetabular cartilage and the total femoral and acetabular cartilage T1 relaxation time. Cam lesion morphology was quantified by two observers measuring the alpha angle on radiographs and reconstructed MRI radial slices at the 12 O'Clock (superior), 1 O'Clock, 2 O'Clock, and 3 O'Clock (anterior) position. Alpha angle measurements were correlated with the dGEMRIC index.
Average alpha angle was 76.8 degrees (range 39.3 to 115.9) on AP radiographs, 62.8 degrees (range 39.7 to 89.3) on cross-table lateral radiographs, and 64.0 degrees (range 33.3 to 116.2) on MRI radial slices. The inter-observer reliability ICC was 0.88. Maximum alpha angle measured on radial MRI correlated best with the dGEMRIC index (r=0.509 p=0.003), followed by alpha angle measured on the cross-table lateral radiograph (r=0.505 p=0.004). Alpha angle measured on an AP radiograph (r=0.333 p=0.067) and the maximum alpha angle on AP and lateral radiographs (r=0.317 p=0.082) correlated less well.
Alpha angle measurements using MRI radial slices of the femoral neck or cross-table lateral radiographs display a superior correlation with the dGEMRIC index than AP radiographs. Longitudinal studies reveal that raised alpha angles on AP radiographs have a low positive predictive value for the development of hip osteoarthritis. The assessment of lateral radiographs or MRI radial slices may improve the predictive value of the alpha angle and prove valuable for patient selection into clinical trials.

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BIOACTIVITY OF PTH 1-34 IN NOVEL NASAL DELIVERY FORMULATIONS FOR THE TREATMENT OF OSTEOPOROSIS

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Introduction

The only anabolic therapeutic agents licensed to treat osteoporosis are PTH 1-34 and PTH 1-84, currently administered via injection. Repeated injections are not well-tolerated by patients, leading to poor adherence, suboptimal dosing, reduced efficacy and increased healthcare costs. A patient-friendly alternative administrative route is an unmet clinical need. Nasal delivery of large peptides requires an absorption enhancer. The aim of this study was to determine osteoblastic bioactivity of PTH 1-34 in formulations with the absorption enhancer Criticalsorb(TM).

Methods

Human osteoblast-like Saos-2 cell mineralisation was demonstrated with alizarin red staining. Saos 2 cells were stimulated with PTH 1-34 alone, standard formulation components or Criticalsorb(TM) formulation. Bioactivity was determined using the well-established PTH receptor canonical second messenger 3'-5'-cyclic adenosine monophosphate (cAMP) pathway whereby PTH 1-34 bioactivity induces cAMP signal. Quantification of cAMP used ELISA (R&D Systems). Cell numbers were normalised using PicoGreen(R) quantification of DNA (Molecular Probes(R), Invitrogen). Data were subjected to statistical analysis. Normality of data was assessed using Kolomogorov-Smirnov and distribution plots. ANOVA was accompanied by Dunnett's post-hoc test. The time post administration of PTH 1-34 to maximal intracellular cAMP peak was measured and applied in all subsequent experiments assessing formulations in vitro bioactivity.

Results

An EC50 of 0.76nM was calculated through titration of PTH 1-34. There was no significant difference in the bioactivity of 10nM PTH 1-34 only and 10nM PTH 1-34 in standard formulations (sodium hyaluronate, poloxamer 407 and chitosan) as determined by intracellular cAMP in Saos-2 cells ($p>0.05$). Criticalsorb(TM)/PTH 1-34 formulation increased the cAMP response 113% (368±72 versus 786±154fmol cAMP/ng DNA respectively, $p<0.05$). Criticalsorb(TM) alone did not stimulate cAMP.

Conclusion and future studies

Criticalsorb(TM)/PTH 1-34 formulation augments PTH 1-34 bioactivity of osteoblasts in vitro. Subsequent ongoing pharmacokinetic research indicates that the formulation enables intranasal uptake of PTH 1-34. Future studies will determine the biological effect of nasal dosing of PTH 1-34 on bone micro-structure and density in an in vivo model of osteoporosis.

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ADIPONECTIN MIMIC, ADP 355, INHIBITS MM CELL PROLIFERATION AND INDUCES APOPTOSIS IN MYELOMA CELLS

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Multiple myeloma (MM) is a haematologic malignancy characterised by the accumulation of monoclonal plasma cells in the bone marrow (BM). Common presenting symptoms of MM are bone pain, and an osteolytic bone disease. Current therapies for myeloma fail to eradicate the disease and the median survival is around 5 years. Adiponectin, an adipokine involved in regulating glucose levels and fatty acid breakdown, has previously been shown to have a negative correlation with MM in vivo and can directly cause MM cell death in vitro. A short peptide mimic of adiponectin, ADP 355 has recently been produced and this study aims to characterise its action in MM cells.
Mouse myeloma cell line, 5TGM1, was used to investigate the effect of ADP 355 in MM. MM cell viability was examined using the MTS assay. Investigation of apoptosis was carried out using western blotting to see the cleavage of apoptosis markers.
MM cells were found to express equal levels of AdipoR1 and R2. ADP 355 induced phosphorylation of AMPK, a known consequence of adiponectin receptor activation. ADP 355 dose-dependently decreased MM cell viability with nanomolar doses of ADP 355 able to inhibit MM cell viability equal to that of adiponectin. Western blotting has shown that ADP 355 increased levels of cleaved caspase-9, a marker of apoptosis. Further analysis of downstream apoptosis markers revealed that cleaved caspase-3 and PARP-1 was also increased with treatment of ADP 355. When the MM cells are cultured with bone marrow stromal cells, treatment of ADP 355 in combination with dexamethasone and bortezomib induced an enhanced effect on the MM cells.
ADP 355 appears to be a functional mimic of adiponectin action in MM cells. The clear increase in apoptosis markers shows that ADP 355 induces cell death in vitro. Further comparative investigation is needed to fully elucidate the function of ADP 355 in vitro and in vivo.

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BONE METASTATIC PROSTATE CANCER CELLS EXHIBIT ADIPOMIMETIC PROPERTIES

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Bone metastases often occur in advanced prostate cancer. The invasion of prostate tumor cells in bone predominantly causes osteoblastic bone lesions, with increased bone mineral density at the lesion site. The precise mechanisms underlying the development and progression of prostate cancer bone metastases are poorly understood. More recently, obesity and adipokines have been associated with an increased risk of developing aggressive prostate cancer. Although adipokines (e.g. adiponectin, leptin) have been shown to increase the motility and the proliferation of prostate cancer cells, their role in prostate cancer bone disease is unknown. There is increasing evidence to suggest that, although originally identified as secreted from adipocytes, adipokines are expressed by a number of different cell types. Furthermore, prostate cancer cells have recently been shown to have the potential to differentiate into adipocyte-like cells. Therefore, we hypothesized that prostate cancer cells may exhibit an adipomimetic phenotype that may contribute to the development of bone metastases.

We used adipokine arrays, PCR and western blotting to assess the expression profile of adipokines in human prostate cancer cells that have high (e.g. ARCaP-M) or low (e.g. ARCaP-E) osteoblastic bone-metastatic potential.
Adipokine array analysis of conditioned media from ARCaP-M cells and ARCaP-E cells revealed differences in the expression level of several adipokines including adiponectin, leptin and leukemia inhibitory factor (LIF). The differential expression of these adipokines was further demonstrated at mRNA level by PCR analysis, with increased expression of these adipokines in ARCaP-M cells as compared with ARCaP-E cells. Higher expression level of adiponectin in ARCaP-M cells was demonstrated by western blotting.
In conclusion, our data for the first time demonstrate a differential expression pattern of adipokines between prostate cancer cells that have high or low bone-metastatic potential, with the finding that adiponectin, leptin and LIF are expressed at a higher level in prostate cancer cells that are more metastatic to bone. The adipomimetic profile of bone metastatic prostate cancer cells suggests that adipokines may play an important role in the development of bone metastases in prostate cancer.

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MOLECULAR EVALUATION AND MICROMECHANICAL ASSESSMENT OF BONE QUALITY

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Elucidation of the factors regulating bone quality has led to candidate material and structural determinants to complement conventional fracture risk measures, such as mineral content, towards fracture mechanics. Though links between bone matrix proteins and mechanical properties and the relationship between bone microenvironment and whole bone mechanics have been recognised, repercussions of molecular to macroscopic level events remain undetermined. The aim of this investigation was to examine bone biology and whether this correlates to bone properties, from the microscale through to whole bone mechanics, to determine new indices of bone quality.
The paradigm was developed using a 140 day old Sprague-Dawley rat model. Osteoblast gene expression was measured from femur epiphyses and whole femurs were used for reference point micro-indentation analysis and micro-CT imaging to investigate associated micromechanics and microarchitecture respectively.
Initial results from the distal femur found correlations between osteogenic gene expression and micromechanical properties. Coefficients between osteocalcin expression and total indentation distance, indentation distance increase and average creep indentation distance were r = -0.74, -0.71 and -0.67 respectively in females, whilst r = -0.86, -0.94 and -0.87 for males (n = 4 for each gender). There were similar supportive findings with RUNX2 for both sexes and osteopontin in females. Micro-CT data of the trabecular bone showed correspondence between osteocalcin and bone volume/tissue volume, fractal dimension and trabecular pattern factor in females (r = 0.82, 0.87 and -0.87 respectively), again this was reflected with RUNX2, osteopontin and collagen I.
These findings provide evidence of improved mechanical properties at the microscale, when osteogenic gene activity is increased in healthy animals, in connection to enhanced trabecular bone structure. There is an inference that bone quality is influenced by the bone biology environment through development of microarchitecture complexity (fractal dimension and trabecular pattern factor), in addition to widely established whole bone strength predictors such as mineralisation. This supports existing work where these structural parameters have been associated to whole bone yield strength. The current approach highlights the potential to correlate molecular and microarchitecture to determine bone quality, with implications therein for diseased skeletal analysis.

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IMPACT BONE IN PERRAULT SYNDROME IN A CASE REPORT

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Perrault syndrome is defined by XX gonadal dysgenesis and sensorineural deafness.Several families with other findings, including neurological, are regularly reported. The impact bone is secondary amenorrhea. We report a case.
observation:
Patient aged 21 years old. With past medical history; congenital intermarriage, deaf-mutism, epilepsy receiving depakine under 5 years. with four similar cases in family, three sisters and a brother with mutism without neurological, and the brother without hypogonadism.
Sent for assessment of bone primary amenorrhea. On examination, there is a impubérisme, a failure to thrive (-3DS), Weight = 39 kg, height = 1.49 m. scoliosis Lumbar with left convexity, no pain or deficit engine, no mental retardation. No anosmia.
Balance sheet: A hypogonadotrophic hypogonadism: FSH-LH high: FSH = 71 IU / L and LH = 20.5 IU / L. Hypocalcemia and hypocalciuria with deficiency vitamin D at 5 ug / l. Bone densitometry is underway. Abdominopelvic ultrasound has not seen ovary and the uterus was hypoplastic. The karyotype was normal. Audiometry objectified profound deafness.
The electro-encephalogram has objectified the presence of bilateral frontal figures with epileptic tendency to generalize on normal background activity. Magnetic reasonnance imaging was normal. The lumbar spine confirmed scoliosis, which was treated with corset and rehabilitation. Supplementation with vitamin D was done.
Conclusion: Our patient illustrat one of rare congenital disease, with endocrinological and bone impact.

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COL1A1 IS NOT ASSOCIATED WITH STRESS FRACTURE INCIDENCE IN ELITE ATHLETES

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Introduction: The occurrence of stress fracture injuries in monozygotic twins (Singer et al., 1990) and multiple stress fractures occurring at various skeletal sites (Lambros et al., 1997) indicate that genetic factors may influence stress fracture susceptibility. The COL1A1 gene encodes collagen type 1, the predominant protein in bone, and has been associated with various bone phenotypes including bone mineral density (Grant et al., 1996), fracture risk (Uitterlinden et al., 1998, Keen et al., 1999) and altered bone metabolism (Keen et al., 1999). However, it is unknown whether COLIA1 is a relevant candidate gene for stress fracture risk in young athletic populations.
Methods: 518 elite (professional or national standard) athletes (449 males and 69 females; age 23±6y; height 1.81±0.12m; body mass 77.96±11.73kg;) from different sports volunteered to provide a saliva sample for DNA analysis and completed a self-reported training and stress fracture history questionnaire. Genotyping for SNP rs180012 within COL1A1 gene was conducted using a proprietary fluorescence-based competitive allele-specific polymerase chain reaction assay (LGC genomincs Herts, UK).
Results: 128 participants reported at least one scan certified stress fracture injuries, 373 participants had no history of stress fracture incidence and 17 participants were removed from the analysis due to inconclusive diagnosis e.g. stress reaction. No significant association between COL1A1 genotype and stress fracture incidence was observed ($P < 0.05$). The SS, Ss, ss genotypes were shown in 71.7%, 25.2%, 3% of non-stress fracture participants and in 72.1%, 27%, 0.8% of stress fracture sufferers. There were also no significant differences in allele frequency ($P < 0.05$), and S and s alleles were observed in 77.5% and 22.5% of the non-stress fracture participants and 78.1% and 21.9% of the of stress fracture sufferers.
Conclusion: Despite evidence for an association between COL1A1 and several bone phenotypes (Grant et al., 1996, Keen et al., 1999), stress fracture injury was not associated with COL1A1 (rs180012) in an elite athletic population. These findings support recent studies in military

personnel (Korvala et al., 2010, Cosman et al., 2013) and suggest that SNP rs180012 is not associated with stress fracture injury.

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REGULATION OF HUMAN MESENCHYMAL STEM CELL ACTIVITY ON NANOSCALE HYDROXYAPATITE / POLY(LACTIC ACID) COMPOSITE SURFACES

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Introduction

Nanoscale hydroxyapatite (HAp) has the capacity to enhance the mechanical and biological properties of polymeric scaffolds for tissue engineering. HAp synthesis can be manipulated to influence crystal morphology, size and crystallinity. This work investigates HAp fabrication, dispersion in a polymeric matrix, material characterization and response of human mesenchymal stem cell (hMSC) to HAp / poly (lactic acid) (PLA) films.

Methods

HAp was produced using a sol-gel method, in brief Ca2+ was titrated into a PO43- solution at room temperature and pH10 was maintained throughout. Dispersion of HAp was optimized using cetyltrimethylammonium bromide (CTAB) and transferred into chloroform. PLA was dissolved in chloroform and mixed with dispersed HAp to a final solid concentration of 2.5% v/v. Composite surfaces were coated on to glass coverslips using a rotary spin coater. HAp and composite surfaces were characterized using a variety of materials analysis. Surfaces were seeded with hMSC for morphology and alkaline phosphatase (ALP) measurement.

Results

Materials analysis revealed an amorphous crystalline with a Ca/P ratio >1.67, crystal planes corresponding to JCPD ref 009-432 and nanoscale-agglomerates with a plate/rod-like morphology. Dispersion of HAp showed agglomerates of <100 nanometers. Surface roughness of spin coated composites varied significantly between PLA (0.072 +/- 0.041 micrometer) and composite surfaces (1.48 +/- 0.786 micometer). SEM confirmed agglomerates of HAp covered by a film of PLA. Similar numbers of hMSC's adhered to both PLA and PLA+HAp surfaces. Cells spread well on both types of surface, but appear to have extended pseudopodia interacting with topographical features created by HAp. ALP activity of hMSC under routine culture conditions for 21 days showed no differences between PLA alone or PLA+HAp.

Discussion

The composite surfaces contained a rough topography influenced by agglomerates of HAp, this could be caused by medium viscosity and Van der Waals forces between HAp crystals during the fabrication process. The absence of significance in ALP activity between PLA and PLA+HAp surfaces could be due obstruction of direct HAp - cell interaction by PLA coating.

Acknowledgements

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MEMBRANE TYPE-1 MATRIX METALLOPROTEINASE (MT1-MMP) IS A POTENTIAL NOVEL THERAPEUTIC TARGET IN OSTEOSARCOMA

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Background

Membrane type 1 matrix metalloproteinase (MT1-MMP) plays a role in the progression of several common solid cancers. HT1080 fibrosarcoma cells express large amounts of this protein and are widely used to study MT1-MMP function. There are no reports regarding MT1-MMP expression in osteosarcoma. Given that osteosarcoma features extensive local invasion and haematogenous metastases, we hypothesised that osteosarcoma cells utilise MT1-MMP to drive these processes.

Aims

1. Examination of MT1-MMP expression in osteosarcoma biopsy tissue in relation to clinical outcome
2. Measurement of MT1-MMP RNA and protein expression in osteosarcoma cell lines
3. Assessment of the effect of MT1-MMP knockdown on osteosarcoma cell invasion

Methods

Immunohistochemistry: Formalin-fixed and paraffin embedded osteosarcoma biopsy samples from 71 patients were immunostained for MT1-MMP and the data correlated with patient survival.

RNA and protein expression: real time PCR and western blotting was performed on a panel of osteosarcoma cell lines, breast cancer cell lines and the HT1080 fibrosarcoma line.

MT1-MMP knockdown: This was achieved using siRNA and confirmed with real time PCR, western blotting and zymography.

Confocal microscopy: demonstrated membranous, cytoplasmic and an unexpected intranuclear presence of MT1-MMP. The Duolink assay revealed co-localisation of MT1-MMP with hypoxia inducible factor-2 alpha (HIF-2 alpha).

Results

Immunohistochemistry showed MT1-MMP immunopositive cytoplasmic and nuclear staining. Biopsy samples with the highest MT1-MMP staining correlated with reduced patient survival.

In vitro studies confirmed elevated MT1-MMP expression levels in osteosarcoma cell lines as compared to the HT1080 fibrosarcoma line and the breast cancer cell lines. MT1-MMP knockdown with siRNA was effective in reducing osteosarcoma cell invasive capability.

Conclusions

MT1-MMP expression in osteosarcoma tissue correlates with patient survival and osteosarcoma cell lines express large amounts of this protein. MT1-MMP knockdown reduces osteosarcoma cell invasiveness. The increased intranuclear presence of MT1-MMP in hypoxia is of particular interest and we have evidence of protein to protein interaction with HIF-2 alpha. Our data indicate a dual role for MT1-MMP: extracellular activity to direct cell invasion and intranuclear localisation in hypoxia, the significance of which is as yet uncertain.

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DELAYED GADOLINIUM-ENHANCED MRI OF CARTILAGE PREDICTS THE PATTERN OF HIP OSTEOARTHRITIS PROGRESSION AT FIVE YEARS

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Femoroacetabular impingement increases the risk of developing hip osteoarthritis, however, morphological parameters have a low positive predictive value. Arthroscopic debridement of impingement lesions is proposed as a potential strategy for the prevention of osteoarthritis, however, such interventions require the identification of individuals at high risk of disease progression. We investigated whether delayed Gadolinium-Enhanced MRI of Cartilage (dGEMRIC) predicts development of hip osteoarthritis. This imaging modality is an indirect measure of cartilage glycosaminoglycan content.

29 asymptomatic individuals (mean age 51 years) from a longitudinal cohort study investigating hip osteoarthritis were assessed at baseline and five years with collection of Patient Reported Outcome Measures (PROMs), anteroposterior and cross-table lateral radiographs, 3D morphological MRI and dGEMRIC of their index hip. A dGEMRIC index was calculated as a ratio of the anterosuperior acetabular cartilage and total femoral and acetabular cartilage T1 relaxation time. Radiographic disease progression was assessed using minimum joint

space width (JSW), lateral sourcil JSW and medial sourcil JSW. JSW measurements were made by a single observer on anteroposterior radiographs with an intra-observer ICC of 0.916. Alpha angle measurements were made by the same observer on radiographs and MRI radial slices with an intra-observer ICC of 0.926.
Mean minimum JSW for the cohort fell by 0.16mm over five years (p=0.024). Baseline dGEMRIC correlated with the direction of JSW loss (change in JSW at the lateral sourcil minus change in JSW at the medial sourcil) (r=0.561 p=0.002) but not change in minimum JSW (r=0.031 p=0.873). There was a weak correlation between the change in Non-Arthritic Hip Score and baseline dGEMRIC (r=0.256 P=0.180). Alpha angle measured on baseline radiographs and MRI radial slices did not correlate with change in minimum JSW and weakly correlated with direction of JSW narrowing (r=0.273 p=0.160).
A low dGEMRIC index indicates reduced glycosaminoglycan concentration in anterosuperior acetabular cartilage compared with total femoral and acetabular cartilage. This correlates with lateral JSW narrowing relative to medial JSW narrowing with osteoarthritis progression. The dGEMRIC index correlates better with osteoarthritis progression than alpha angle measurements and offers potential to refine a predictive model for disease progression to aid patient selection for clinical trials.

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DISTAL FEMORAL FRACTURES: 5 YEAR MORTALITY AND SECULAR TRENDS

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Background and Objectives: Distal femoral fractures are 10 times less common than hip fractures. 12-month mortality has been reported as 25-30% but there is no longer-term data. In Northumbria hip fractures have a 5-year mortality of 68%. The aim of this study is to analyse 5-year mortality in distal femur fractures in the Northumbrian NHS trust, and identify risk factors for mortality. To compare the results to literature standards and Northumbrian hip fracture data.
Methods: This retrospective observational study included patients admitted with distal femur fractures (AO type 3.3), including periprosthetic fractures, between 01/01/05 and 31/12/07. Patient information, which included age, gender, co-morbidities and date of death, was collected through hospital coding and analysis of notes. Co-morbidity data was only available for 53 patients. Mortality rates were calculated, and stratified according to age, gender and co-morbidities. The results were analysed using backwards-multivariate linear regression to determine the significance.
Results: 83 patients (74 female) were identified and the population had a mean age of 80 years (range 60-102). The overall 5-year mortality was 72%. Mortality increased with age and being female may be a risk factor. Dementia and COPD were the only statistically significant predictors of mortality.
Conclusion: This study shows an overall 5-year mortality of 72% in patients over 60 with distal femur fractures. It shows that Dementia and COPD are the most important predictors of mortality. Furthermore, 5-year mortality was higher than that of hip fractures in Northumbria, highlighting the vulnerability of this patient group.

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CONTROLLING MICROPOROSITY IN BIOCERAMIC SCAFFOLDS FOR TISSUE ENGINEERING APPROACHES IN BONE

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Background and objectives
Apatite/Wollastonite (A/W) is a glass ceramic that combines biocompatibility, bioactivity, osteoconductivity and bioresorbability(1) and can be used as material for bone scaffolds. It is well established that implant microstructure impacts on the relationship of the scaffold with surrounding cells and tissues influencing treatment success. Therefore, in this study two fabrication approaches were contrasted to assess the resulting microstructure and physical parameters of A/W scaffolds. The first group of scaffolds was fabricated by ceramic powder casting in moulds followed by sintering. The second group was fabricated combining thermally induced phase separation and sintering.
Methods
A/W glass was produced by GTS, Sheffield, UK as reported by Kokubo(1).
Powder casting and sintering: A/W powder with different particle fractions (20-53 micrometers and 54-90 micrometers) was poured in platinum tubes and then sintered at 1150 degrees Celcius.
Thermally induced phase separation (TIPS) and sintering: PLA was dissolved in Dioxane-1,4 and after complete dissolving of PLA A/W powder (20-53 micrometers) was introduced in the solution. The slurry was stirred at 40 degrees Celcius for 2h and then, poured into cylindrical moulds. The scaffolds were kept overnight at low temperature and then the dioxane was washed off in aqueous ethanol. The scaffolds were then freeze dried, before being sintered at 1150 degrees Celcius.
Scaffolds were then analysed by SEM, image J, Archimedes method and micro-CT.
Results
It was found that the powder casting method and sintering resulted in constructs with pore size <10 micrometres and overall porosity levels around 30%. The scaffolds made with TIPS and sintering had pores around 100 micrometres and 80% porosity.
Conclusion
Both methods are able to produce microporous scaffolds over a range of processing conditions. Scaffolds made using TIPS and sintering have higher microporosity and interconnectivity in comparison to the sintered powder casted scaffolds. Microporosity affects cellular activity and differentiation, so these scaffolds are going to be assessed using human mesenchymal stem cells to evaluate the optimal microporosity that induces bone formation.
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DO MODERN TOTAL KNEE REPLACEMENTS OFFER BETTER VALUE FOR MONEY? A HEALTH ECONOMIC ANALYSIS

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Purpose
Cost effectiveness is an increasingly important metric in today's healthcare environment, and decisions surrounding which arthroplasty prosthesis to implant are not exempt from such health economic concerns. Quality adjusted life years (QALYs) are the typical assessment tool for this type of evaluation. Using this methodology, joint arthroplasty has been shown to be cost effective, however studies directly comparing the QALY achieved by differing prostheses are lacking.
Methods
Data was gathered in a single centre prospective double-blind randomised controlled trial comparing the outcome a modern implant, the Triathlon total knee replacement, with its traditionally designed predecessor the Kinemax, using the Short Form 6 dimensional (SF-6D) score and quality adjusted life year (QALY) methodology. The study cohort consisted of 64 patients that were randomised to a Triathlon and 60 randomised to a Kinemax.
Results
There was a significant improvement in the SF-6D score for both groups at one year compared with pre-operative scores (p<0.0001). The calculated overall life expectancy for the study cohort was 15.1 years, which resulted in an overall QALY gain of 2.144 (95% CI 1.752 - 2.507). The modern implant group demonstrated only a small improvement in the SF-6D score compared to the traditional design at one year (0.141 versus 0.143, p=0.94). This difference in health gain

resulted in the modern implant costing £298 less per QALY at one year, however this saving diminished to less than £30 per year over the lifetime of the cohort.

Discussion

This study demonstrates that despite comparing 2nd and 4th generation design, modern implant technology does not influence the cost-effectiveness of TKA using the SF-6D and QALY methodology. As most implants demonstrate similar longevity, differences in patient function will likely carry the greatest influence on QALY. This type of analysis however assesses health status, and is not sensitive to joint specific function. Dramatic differences in patient outcome would be required to influence QALY score. Evolutionary design changes in implant technology are thus unlikely to influence QALY analysis following joint replacement, which has important implication for implant procurement.

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REJUVENATION OF MESENCHYMAL STROMAL CELLS THROUGH CONTROLLED AUTOPHAGY

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The skeletogenic differentiation potential of mesenchymal stromal/stem cells (MSCs) makes them attractive therapeutic cells for bone disorders. However, the quality and efficiency of MSC differentiation, particularly from aged patients, is clinically inadequate so there is a requirement for developmentally younger, safe, autologous cell sources. We hypothesised that cytoplasmic and organelle restructuring through autophagy (a survival response to nutrient depletion and removal of aged/defective organelles) accompanied by bioenergetic transition, can drive reprogramming towards a primitive ground state; effectively mimicking the metabolic and uncommitted characteristics of embryonic stem cells (ESCs). To test this hypothesis, we established conditions to generate variations in nutrient access to scale the autophagic response by growing human MSCs as 3D spheroids in a range of sizes and culture periods. Screening for pluripotency features, we demonstrated that expression of Oct4, Nanog, Sox2 and telomerase increased significantly at day 5 using 60,000 MSCs, compared to 2D MSC controls. In addition, 3D MSCs were enriched in early mesodermal/hemangioblastic markers (CXCR4, MCAM, PECAM, PAX2, MSX1, SNAI1, LHX1) with loss of differentiated-related transcripts (COL1A1, POST, OGN, OMD, ACAN, HAPLN1) and significantly increased (20-4000-fold) expression of MLC, MHC and MEF2C under cardiomyogenic induction protocols, implicating a reversion to a less restricted mesodermal precursor. Enhanced potency corresponded with increased TFEB, LC3 lipidation and LAMP1 levels; evidence for advanced autophagy and cellular remodelling. Extensive autophagosomal organelles and regression to immature perinuclear mitochondria were also observed, accompanied by a shift from oxidative to glycolytic metabolism, without compromising cell survival. Pluripotency factor expression was accelerated when exposed to rapamycin, a stimulator of autophagy. Importantly, following subcutaneous implantation into immunocompromised mice, 3D MSCs did not form teratomas (as in ESC positive controls) but spontaneously generated musculoskeletal tissues including fat and muscle, confirming enhanced potency and suggesting a response to host cell signalling; 2D MSCs were without effect. Following disaggregation, 3D MSCs could be expanded in semi-solid medium, to retain diminished cell size and elevated Oct4, Nanog, Sox2, telomerase and TFEB expression. These data demonstrate a novel, rapid and efficient mechanism to rejuvenate MSCs from aged donors that can be exploited for effective restoration of tissue function in bone disease.

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THE ASSOCIATION BETWEEN BASELINE BETA-CTX CONCENTRATIONS AND BETA-CTX RESPONSE TO 2 HOURS OF TREADMILL RUNNING

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Introduction: The reported bone resorption response to strenuous exercise is variable, with the BETA-CTx response showing a high degree of individuality. Low participant numbers, feeding (Scott et al., 2012), exercise intensity (Scott et al., 2010 and, most likely, duration may all influence this response. The present study aimed to examine the BETA-CTx response to 2 h of running at 70% VO2max and to determine whether this response is influenced by baseline BETA-CTx values.

Methods: 32 recreationally active males (age 23±3 y; height 1.79±0.04 m; body mass 77.1±8.8 kg; VO2max 55.4±5.9 ml.kg-1BM.min-1) were recruited. Participants performed a fasted 2 h run at 70% VO2max after a 3 d controlled lead in period. Blood was taken at baseline (08:30, prior to a 09:00 run), immediately following, 24, 48 and 72 hours post run and analysed for Beta-CTx using serum Cross Laps (CTX-1) ELISA (Immuno Diagnostic Systems).

Results: There was no significant overall effect of 2 h of treadmill running on serum BETA-CTX concentrations. BETA-CTx concentrations were 0.77±0.28 (µg.L-1) at baseline and following 2 hours of treadmill running were 0.71±0.27 (µg.L-1), with 24, 48 and 72 h post-exercise concentrations being 0.76±0.36 µg.L-1, 0.82±0.37 µg.L-1 and 0.80±0.39 µg.L-1. Baseline Beta-CTx was significantly correlated with the change in Beta-CTx immediately following 2 h treadmill running (Correlation .47; $P>0.05$), but not with the three subsequent follow-up days ($P<0.05$).

Conclusion: 2 hours treadmill running did not affect bone resorption, as determined by Beta-CTx concentrations, although a degree of individual variability was observed in this response. Higher baseline Beta-CTx concentrations were correlated with a greater decrease in post run Beta-CTx concentrations immediately following 2 h treadmill running, but no correlation was seen in the three follow-up days. The correlation between baseline Beta-CTx concentrations and acute Beta-CTx response to exercise may explain, in part, the variability seen in the Beta-CTx response to exercise. Further research is required to elucidate the reasons (e.g. genetics, assay differences, diet) for the individual bone resorption response to acute exercise.

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DIFFERENTIAL EFFECTS OF NITROGEN-CONTAINING BISPHOSPHONATES ON HUMAN PBMCS AND MUTZ-3 CELLS

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Introduction: Nitrogen-containing bisphosphonates (N-BPs) can inhibit the differentiation and function of osteoclasts derived from Peripheral Blood Mononuclear cells (PBMCs) in a dose-dependent manner. MUTZ-3 cells are a potentially useful human cell line for studying osteoclast differentiation. The aim of this study was to elucidate the action of N-BPs on MUTZ-3 cells.

Methods: Human PBMCs and MUTZ-3 cells were cultured in alpha-MEM supplemented with heat inactivated foetal calf serum, 25ng/ml hMCSF, 100 ng/mL soluble hRANKL and 25ng/ml hTNF-alpha. To determine the effects of BPs, cells were cultured in the presence of alendronate, ibandronate, risedronate and zoledronate at concentrations ranging from 10nM to 50µM. The extent of osteoclast formation (expressed as the number of TRAcP+ multinucleated cells) as well as cell morphometry of newly-generated osteoclasts (osteoclast area and numbers of nuclei/osteoclast) were also determined. The osteoclast markers cathepsin-K, calcitonin receptor (CTR) and osteoclast-associated receptor (OSCAR) were demonstrated by western blotting. Experiments were performed in the presence of inhibitors of cell internalisation and fluorescent FAM-Risedronate to determine the pathway(s) by which these cells internalise BPs.

Results: Multinucleated osteoclast-like cells were evident at day 12 and the osteoclast markers, cathepsin-K, CTR and OSCAR were detectable from day 12 in both cell populations. Analysis of N-BP treatment compared with controls revealed significant dose-dependent effects

only in PBMC cultures. Although N-BPs altered osteoclast area and number of nuclei/osteoclast in PBMC cultures, these drugs failed to induce such effects with MUTZ-3 cells. Sodium vanadate, a specific inhibitor of ATPase-dependent endocytosis blocked internalization of N-BPs in PBMCs, whereas caffeine, a specific inhibitor of Ca2+-dependent endocytosis, blocked uptake by MUTZ-3 cells.
Conclusion: Although MUTZ-3 cells can differentiate into TRAP-positive multinucleated osteoclast-like cells which express osteoclast markers, these cells do not respond to BPs in a similar manner to PBMCs, even though BPs can be internalised by both cell types. The reasons why MUTZ-3 cells are unresponsive is so far unexplained.

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PLACENTAL SIZE AND COMPOSITION ARE ASSOCIATED WITH OFFSPRING SKELETAL DEVELOPMENT IN 9 YEAR OLD BRITISH CHILDREN

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Introduction: We have previously shown that placental volume is associated with offspring bone size at birth. It is not known whether such associations persist into later childhood and whether there might be differential placental determinants of bone size and density. We examined associations between placental morphology and childhood bone size and density in a population-based mother-offspring cohort.
Materials and Methods: The Avon Longitudinal Study of Parents and Children (ALSPAC) recruited women from the former region of Avon, UK. From April to December 1991, 12,942 babies were born at term and their placentas were preserved in formaldehyde. At 9 years the children underwent a DXA scan in order to assess whole body minus head bone area (BA), bone mineral content (BMC),areal bone mineral density (aBMD) and size-corrected BMC (BMC adjusted for BA, height and weight). In 2010 a sample of 1,680 placentas were measured and photographed. Placental length, width, thickness, weight and the number of cotyledons were recorded.
Discussion: Placental volume predicted BA (0.14 SD/SD, p=<0.001), BMC (B=0.12 SD/SD, p=0.0001) and aBMD (B=0.08 SD/SD, p=0.02) after adjusting for child age and sex. The ratio of cotyledons to volume predicted size-corrected BMC (B=0.09 SD/SD, p=0.01) after adjusting for age and sex.
Conclusion: These results demonstrate that previously observed positive relationships between placental volume and offspring neonatal bone size persist into later childhood. Additionally, the positive relationship between placental cotyledon to volume ratio and size-corrected BMC suggest a possible differential effect of placental size and architecture on childhood bone size and volumetric density.

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THE ROLE OF SCARA-5 IN BONE PHYSIOLOGY AND PATHOLOGY

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Background
The tumour suppressor gene Scavenger Receptor Type-A Number 5 (SCARA-5) is down-regulated by promoter hypermethylation in cancer cell lines [1,2] and heavily up-regulated during adipogenesis [3] and glucocorticoid treatment of osteoblasts [4]. As the prototypic member of the Scavenger Receptor superfamily - SR-A - is a negative regulator of bone density through osteoclast activation [5], we sought to investigate the effects of SCARA-5 inhibition during bone homeostasis and disease.
Materials and Methods
Immunohistochemistry of healthy and damaged regions of osteoarthritic femoral and tibial head biopsies was used to show expression of SCARA-5 during bone remodelling. Micro-CT analysis of SCARA-5-/- and wild-type (WT) adult mice was undertaken to compare macroscopic effects on bone histomorphometry. Bone Marrow (BM) was flushed from the long bones of these mice: Osteoclastogenesis of WT and transgenic animals was compared by culturing osteoclast precursors on dentine slices and subsequent staining with toludiene blue. Flushed cells were also expanded and osteoblastogenesis of each compared by culturing in osteogenic media for 3 weeks; mineral deposition was compared by Alizarin Red staining.
Results and conclusions
Immunohistochemical analyses confirmed the expression of SCARA-5 in human bone and showed an up-regulation in regions of osteoarthritic damage. Morphometry of the trabecular bone beneath the tibial growth plate showed increased bone surface/volume in knock-out (k/o) mice with increased trabecular number and connectivity. In vitro studies showed SCARA-5 acts as a suppressor of osteoblastogenesis as BM cultures from k/o animals had significantly increased mineralization over WT mice, with no apparent effects on dentine erosion.
These results indicate for the first time that SCARA5 is a negative regulator of bone density and that it may be a therapeutic target for reducing the osteolysis associated with osteoarthritic progression.
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INFLUENCE OF ETHNICITY ON BONE MINERAL DENSITY AND HIP AXIS LENGTH IN UK MEN

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Understanding how bone structural parameters differ between ethnic groups may provide insight into how differences in fracture risk occur. The aim of this study was to characterise bone mineral density (BMD) and hip axis length (HAL) in three ethnic groups of UK men.
Men aged 40-79 years of European origin (E) were recruited in South Manchester for participation in a study of ageing: the European Male Ageing Study. An additional sample of men of African-Caribbean (AC) and South Asian (SA) origin were recruited from the Manchester area. All subjects attended for dual energy X-ray absorptiometry (DXA) of the hip (femoral neck and total) and lumbar spine (L1-4) and measurement of height and weight. Differences between ethnic groups were assessed using linear regression with DXA parameters (including HAL) as dependent variables with adjustments made for age, height and weight. The results are expressed as mean absolute values and 95% confidence intervals.
339 European (mean age 60.8, SD 11.0), 64 South Asian (mean age 59.2, SD 11.0) and 43 African-Caribbean (mean age 59.6, SD 11.4) were included in the analysis. Femoral neck BMD was significantly higher in AC (0.928 [0.891, 0.965] g/cm2) compared to both SA (0.854 [0.823, 0.886] g/cm2) and E men (0.818 [0.804, 0.831] g/cm2), with a significant difference also between SA and E men. Total hip BMD was also significantly higher in AC (1.149 [1.110, 1.188] g/cm2) compared to both SA (1.049 [1.016, 1.082] g/cm2) and E men (1.014 [1.000, 1.028] g/cm2), however the difference between SA and E men was not statistically significant. AC men had a significantly higher lumbar spine BMD (1.140 [1.089, 1.191] g/cm2) compared to the other groups, with no significant difference between SA (1.069 [1.026, 1.112] g/cm2) and E men (1.073 [1.055, 1.092] g/cm2). HAL was significantly shorter in both AC (118.4 [116.4, 120.4] mm) and SA (118.4 [116.7, 120.1] mm)

compared to E men (123.8 [123.1, 124.5] mm), with no difference between AC and SA men.
There were differences in BMD and HAL between ethnic groups of men living in the UK and these differences persisted after adjustment for body size.

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EFFECT OF INCREASED FRICTIONAL TORQUE ON THE FRETTING CORROSION BEHAVIOUR OF THE LARGE DIAMETER FEMORAL HEAD: AN IN VITRO STUDY

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High failure rates with large diameter metal on metal hip replacements have highlighted the taper / trunnion junction as a potential source of metal ion release. Postulated causes include mechanical wear from micromotion and fretting corrosion. This study investigated the relationship between increasing frictional torque and fretting corrosion for large diameter heads.
36mm Cobalt Chrome (CoCr) femoral heads were coupled with either a CoCr or Titanium (Ti) stem with 12/14 tapers. Increasing perpendicular horizontal offsets created incremental increases in torque. Offset increments of 0mm, 5.4mm and 7.5mm were selected to simulate the torque force equivalent to 28mm, 36mm and 50mm diameter femoral heads. An inverted hip replacement setup was used (ASTM F1875-98). Components were statically loaded at 0kN and 2.3kN prior to sinusoidal cyclic loading and electrochemical testing. Mean currents & fretting currents (peak-trough current) were calculated every 50 cycles up to a maximum of 1000 cycles along with the Overall Mean Current (OMC), Fretting Current (OMFC) and Current change (OCC).
There was a significant increase in the mean (R=0.992, p=0.008) and fretting current (R=0.929, p=0.071) for CoCr-CoCr and in the mean (R=0.780, p=0.005) and fretting current (R=0.810, p=0.006) for CoCr-Ti material combinations, with increasing femoral offsets. The highest currents (mean and fretting) were produced at 7.5mm and the lowest at 0mm offsets. The proportional relationship between torque and corrosion was observed for both CoCr-CoCr and CoCr-Ti material combinations. With low torques we saw higher OMC and OMFC with the Co-Ti material combination however with higher torques we saw higher OMC and OMFC with the CoCr-CoCr combination.
Increasing torque leads to increased susceptibility to fretting corrosion at the modular head-stem taper interface of total hip replacements for both head stem material combinations. This study highlights the risk of high frictional torque, independent of material combination, on the taper / trunnion with the use of large heads. This is particularly relevant with the increasing use of larger diameter femoral heads across all bearing material combinations, in current hip arthroplasty practice.

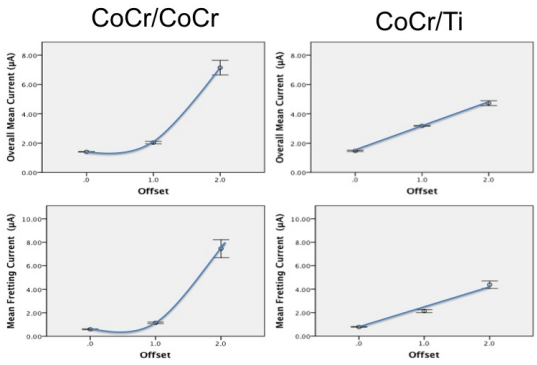

Figure 1: Increasing torque leads to increased susceptibility to fretting corrosion at the modular head-stem taper interface of total hip replacements for both head stem material combinations. At higher torques both the mean and fretting corrosion is higher for the CoCr-CoCr material combination. At lower torques both the mean and fretting corrosion is higher for the CoCr-Ti material combination.

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MEASUREMENT OF MECHANICAL PROPERTIES THROUGHOUT PORCINE TIBIAL PLATEAUX AS A MECHANISM TO UNDERSTAND EARLY OSTEOARTHRITIS

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Introduction
Osteoarthritis (OA) is one of the ten most disabling diseases in developed countries. OA of the knee is one of the most common types of OA. It has been observed that unicompartmental knee OA occurs with very distinct and repeatable lesion patterns [1].
It is hypothesised that these patterns are the result of differences in the material properties throughout articular cartilage. The aim of this study was to measure the mechanical properties of porcine cartilage in a whole undamaged tibial plateau (TP).
Materials and Methods
Five porcine TP were scanned using a high resolution laser to obtain the topography. Using a custom program, a grid of equally spaced points (6 mm) was defined.
In vivo loading for daily activities occurs normal to the surface [2], therefore indentation was carried out on the same orientation. The normal vector for each indentation point was calculated by averaging the normal vectors of the points within the contact area at full load. The resulting vector allowed the calculation of angles, rotations and translation to obtain normal indentation of each point.
Using a novel whole articular surface indentation machine (WASIM) in combination with a custom program, the TP was rotated to obtain normal indentation. Displacement controlled indentation was performed at 10 percent per second (pps) to 15% of the total cartilage thickness.
Elastic modulus (Em) was calculated at each indentation point by using Hertz contact theory and the Field and Swain Method. It was assumed that the initial portion of the unloading was purely elastic.
Results and Conclusions
Em of 45 to 50 points throughout the TP were obtained for each knee. Results show low Em values in the anterior medial area. Additionally, it was possible to find an area in the posterior lateral section of the TP delimited with low Em values. These areas correspond to the unicompartimental knee OA lesions. These results suggest a correlation exists between the material properties of the TP and the locations of early lesions in knee OA.
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ARTIFICIAL ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION FOR MORE NATURAL KNEE KINEMATICS

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Aim: The aims of this study were to define the design criteria of an artificial ACL which could reproduce the non-linear load-elongation characteristics of the native ACL, and to investigate the mechanical behaviour of a novel ACL reconstruction design consisting of a metallic elastic system and a polymeric cord.
Introduction: Kinematic and survivorship studies on the ACL intact and ACL deficient knees have emphasised the importance of preserving and/or reconstructing the ACL [1], [2]. The unique mechanical properties of the ACL and the non-linear relationship between the ACL forces and the quadriceps muscle forces at different flexion angles are the key elements in providing normal kinematics. Current synthetic ACL reconstruction grafts have shown poor long-term results, mainly due to wear, creep, fatigue and mechanical failure. None of the synthetic and biological grafts used for the ACL reconstruction have been able to

replicate the normal mechanical behaviour of the ACL and prevent degenerative disease progression such as osteoarthritis.
Method: Desired mechanical properties of the artificial ACL were defined based the results of in vitro and in vivo biomechanical studies. Suitable materials were found for the prosthetic ACL which met the required design criteria. Implicit finite element analyses were performed on a spring-cord construct design and the output force-elongation data compared to the estimated in vivo natural ACL properties found in the literature.
Results: It was shown that an artificial ACL should have a non-linear stiffness with low resistance to the initial load (~30 Nmm-1 stiffness in the toe region) and increased stiffness under higher load (~110 Nmm-1 stiffness in the linear region). Suitable materials for the ACL reconstruction design (i.e. CoCrMo alloy and UHMWPE fibres) were identified based on their biocompatibility, strength, strain, creep and fatigue properties. The FEA results showed that the mechanical properties of the novel artificial ACL deisgn closely resembled that of the natural ACL at 30 degrees of flexion. A validation test was performed on prototype samples, which supported the finite element data.
Conclusions: The non-linear force-elongation properties of the native ACL can be reproduced by an artificial ACL reconstruction system in the ACLD knees.
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VERTEBROPLASTY AS A SUCCESSFUL PAIN RELIEF MODALITY FOR COMPRESSION FRACTURE IN ANKLYOSING SPONDYLITIS: A CASE REPORT

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Ankylosing spondylitis (AS) is a chronic inflammatory disease with multiple extra-articular manifestations. Osteoporosis is a known complication of the disease. Vertebral compression fractures in AS is multifactorial in aetiology. Vertebroplasty (VP) is mode of therapy that primarily offers pain relief in patients with acute osteoporotic vertebral fractures with few complications. It is a minimally invasive procedure which has been shown to be effective and safe. To our knowledge, there have been no reported cases of the use of VP to treat an acute verterbral compression fracture in a patient with AS.
We report a patient with AS and multiple cardiovascular co-morbidities who underwent VP of the L1 verterbra fifteen weeks after the initial fracture. The indication for the procedure was intractable back pain leaving him largely bedbound despite the use of multiple analgesics. Although he was offered surgery, he was deemed too high risk to safely proceed by the anaesthetists in view of his multiple co-morbidities. Intraoperatively, no complications were noted. The patient reported an improvement in pain control immediately after, and was able to ambulate independently hours after the procedure. Improvement in SF 36 scores immediately and one month post procedure were seen.
In conclusion, vertebroplasty can successfully provide pain relief in osteoporotic compression fractures in patients with AS. It has a generally low complication risk, and can be performed under local anaesthesia with a relatively short procedure time. One drawback of the procedure is possible non-union of the fracture. The stabilisation provided by vertebroplasty is biomechanically insufficient by itself in the long term. We recognise the need for larger scaleprospective cohort studies and longer follow- up times in order to more accurately determinethe success of pain relief and safety of vertebroplasty in AS pts. Similar studies to assess the incidence of adjacent level fractures in the spine of AS patients following vertebroplasty are also needed. We still suggest close and regular follow-up consultations after vertebroplasty in AS patients in order to increase the detection rates of new fractures following the procedure.

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POSTERIOR EDGE LOADING SIMULATION A NEW METHODOLOGY

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Edge loading is observed in the majority of hip retrieved implants, whether CoC or MoM [Esposito ISTA 2012]. Most of these can be determined to occur posteriorly (rather than antero-superiorly). Although the yearly measured wear rate is higher for antero-superior wear, the in vivo frequency of activities leading to this loading scenario being lower suggest a relatively severe wear regime. Antero-superior wear can be simulated using a standard wear simulator. Posterior edge loading simulation is more challenging, more particularly if impingement is included. To this effect, the use of a robot was investigated. These systems offer full freedom in loading scenarios, and therefore a potential solution for full load spectrum verification approaches in orthopaedics.
An ABB robot (ABB Ltd, Zurich), based at the Fraunhoffer Institute in Germany was used. SolidWorks SP4.0 (Dassault Systeme SA) was used to determine the robot arm positioning. The acetabular cup component was mounted into a Sawbones block, itself mounted in a force plate apparatus (AMTI-OR6-7-2000). The force plate was used in an attempt to investigate the torques generated within the bearing. During initial verification, internal/external motions were simulated, with a modular head mounted on a rigid fixture fixed to the robot arm. A 1kN axial load was applied. Posterior edge loading was simulated using CAD software for a 48mm hard bearing with the acetabular cup at a simulated 45 degrees of abduction with a 5 degree anteversion. The head was mounted onto a Sawbones femur. The tests were run in calf serum solution (25%) with a 1kN axial load.
The internal/external torque generated was 1Nm, in good agreement with tests previously performed on a friction simulator type of apparatus. The robot could be programmed to simulate the posterior edge loading scenario. Further work is required to fully assess the potential of this methodology.

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PREDICTING THE FRACTURE GAP MOTION OF PLATED BONE; WORKING TOWARDS PATIENT SPECIFIC CLINICAL WEIGHT-BEARING GUIDELINES

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When using internal fixation plates as bridging devices, such as is the case with locking plates, it is known that the movement at the fracture site is governed by many factors including: plate dimensions, bone-plate off-set and the positioning of the screws within the plate. Additionally the length of the bone and the fracture location can also influence movement. Many studies have examined methods of increasing the mechanical performance of plating such as fatigue strength and pull-out resistance; fewer studies evaluate ways to produce a more osteoconductive environment at the fracture site. To our knowledge no study has yet provided quantitative guidelines on producing optimal fracture site motion. This requires being able to predict the motion produced for a given device configuration in a given fracture pattern at a given load - all patient or device specific parameters. This study aimed to develop empirical relationships for fracture gap motion prediction based on simulation results.
The study initially focussed on tibial diaphyseal fracture patterns. Three-dimensional finite element and analytical models were developed to evaluate the fracture gap motion under axial loading in selected screw positioning scenarios. The influence of selected variables was examined: choice of screw placement, size of the fracture region and plate dimensions.
The simulation results showed that both the working length of the plate (the distance between the two inner most screws) and the level of applied load are non-linear determinants of fracture gap motion. The motion was also sensitive to the plate geometry. The relationships developed provide quantitative information for predictions of fracture gap motion. We plan to further these results for more scenarios to produce comprehensive empirical guidance for clinical patient weight bearing guidelines.

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DETECTION AND QUANTITATION OF SUBCHONDRAL PROTRUSIONS IN CADAVERIC FEMORAL HEADS BY MR IMAGING

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There is a growing realisation that age-related joint degeneration is a complex, multifactorial disease of the whole joint rather than solely a loss of cartilage. At least some forms of osteoarthritis (OA) appear to be initiated at the subchondral plate. The aim of this study was to investigate the microanatomy of the bone-cartilage interface using MRI and to correlate structural changes with age and degeneration.

A series of six femoral heads obtained from bequeathed cadavers (age 30-96y) were subjected to MRI using a wrist coil on a Trio 3 Tesla instrument. Isotropic voxel data were acquired using a range of different modalities including dual echo steady state (DESS). Images were analysed using Image J and 3D reconstructions made with AMIRA.

In all the samples examined, there was evidence of micro-anatomical lesions arising from the subchondral plate and protruding into the hyaline cartilage, but not extending to the articular surface. The lesions were present as areas of low signal yield, silhouetted against signal-rich articular cartilage. Optimum identification was made using DESS sequences; other sequences were less defined and variable. Protrusions were only counted if they were a) present in multiple sequential slices on DESS at 0.23mm resolution, b) the area on a slice was greater than 3mm2, c) they arose from the subchondral plate and d) were contained entirely within HAC. Within this cohort, the highest number of protrusions, 21, was found in the sample from the oldest donor (96y) and the least, 8, was found in the youngest (30y).

This study reveals that subchondral protrusions are widely found in human femoral heads and that their incidence increases with age. Experiments are on-going to determine if they are similar to the hypermineralised structures first discovered in metacarpal condyles of racehorses with palmar osteochondral disease (Boyde et al 2011), and subsequently in a human femoral head from an alkaptonuia patient with OA. These structures appear to be formed by extrusion of mineralisable matrix through microcracks in the articular calcified cartilage. We propose that they could contribute to the mechanical destruction of cartilage and could provide an imaging biomarker of OA.

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RELATIONSHIPS BETWEEN AGE, SEX AND BONE MICROARCHITECTURE AT THE DISTAL RADIUS AND TIBIA IN LATE ADULTHOOD

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Bone health deteriorates with age but exact relationships are thought to vary between men and women. High resolution peripheral quantitative computed tomography (HRpQCT) scanners have permitted the direct and indirect non-invasive assessment of bone macro- and micro-architecture, including detailed delineation of cortical and trabecular structure. We used this new technique to explore differences in bone according to age and sex in a large, well-characterised cohort of older adults.

198 men and 178 women from the Hertfordshire Cohort Study born between 1931 and 1939 were studied. HRpQCT (Xtreme CT, Scanco Medical) images (voxel size 82micrometres) of the non-dominant distal radius and tibia were acquired. Standard morphological analysis was performed for assessment of macrostructure, density, cortical porosity and trabecular microarchitecture.

The mean(SD) age of participants was 76.1(2.5) and 76.5(2.6)years in men and women respectively (range 72.1-81.4years). At both radius and tibia, men had greater bone area (cortical, trabecular, total); trabecular volumetric density; and trabecular number than women (all $p<0.001$). Trabecular thickness was greater in men than women at the radius ($p<0.001$) but not the tibia ($p=0.2$). Conversely, cortical volumetric density was greater in men in the tibia ($p<0.001$) but did not differ at the radius ($p=0.8$). Radial cortical porosity was higher in men whereas tibial cortical porosity was greater in women. In the radius and tibia of women, there was a negative association between age and both cortical area and thickness, and a positive association with trabecular area. In this group we also observed an inverse association between age and both cortical and trabecular density at both sites ($p<0.05$). Furthermore, trabecular number and thickness were lower in older women, particularly in the radius. These relationships were not seen in men.

In summary, using HRpQCT techniques to ascertain bone macro- and microarchitecture, we have confirmed consistently greater bone areas in men than women. Conversely, sex differences in trabecular microarchitecture and cortical density and porosity appear to be site specific. In this cross-sectional study, we noted an age-difference in cortical and trabecular density, trabecular microarchitecture, and bone geometry at both sites in the eighth decade among women; no such difference was observed in men.

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ASSOCIATIONS BETWEEN BIRTH WEIGHT AND BONE MICROARCHITECTURE IN THE RADIUS AND TIBIA OF OLDER ADULTS FROM THE HERTFORDSHIRE COHORT STUDY

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Evidence is accruing that environmental factors in early life have a critical influence on the magnitude of peak bone mass achieved, and on later risk of fracture. To date, no studies have investigated the relationship between birth weight and bone microarchitecture in human populations. High resolution peripheral quantitative computed tomography (HRpQCT) scanners permit the non-invasive assessment of cortical and trabecular structure; we used HRpQCT to investigate the relationship between birth weight and bone macro- and micro-architecture and volumetric BMD (vBMD) in older age in the Hertfordshire Cohort Study.

198 men and 178 women born between 1931 and 1939 were studied. Birth weight was obtained from birth records. Ages at the time of scanning ranged from 72.1 to 81.4 years. HRpQCT images (voxel size 82micrometres) of the non-dominant distal radius and tibia were acquired with an Xtreme scanner (Scanco Medical). Standard morphological analysis was performed for assessment of macrostructure, vBMD, cortical porosity and trabecular microarchitecture. Anthropometric measurements were taken and information on demographics, lifestyle, and comorbidities were obtained from study questionnaires.

The mean(SD) age of participants was 76.1(2.5) and 76.5(2.6) years in men and women respectively. There was a positive association between birth weight and bone area (total and trabecular) in men and women at both the radius and tibia ($p<0.05$). In women, birth weight was negatively associated with trabecular BMD (Beta(95%CI) radius -16.8(-29.4,-4.2), tibia -12.5(-24.3,-0.8)mg/cubic cm/kg) and trabecular thickness (Beta(95%CI) radius -3.47(-6.63-0.32), tibia -6.09(-9.56,-2.63)micrometres/kg) ($p<0.05$ for all). With the exception of radial trabecular thickness, these associations were robust to adjustment for adult height and weight. There was no evidence of an association between birth weight and cortical area, vBMD or porosity in either sex.

In summary, we have observed relationships between early life and bone area in both men and women in their eight decade. Associations between birth weight and trabecular architecture were identified in women and all but radial trabecular thickness were maintained after adjustment for body size. This may suggest an oestrogen dependent effect. Further work in larger groups is indicated to reproduce these findings, and to relate their significance to fracture incidence.

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COLLAGEN PACKING IN OSTEOARTHRITIC AND OSTEOPOROTIC BONE: A NEUTRON DIFFRACTION STUDY

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Osteoarthritis (OA) and osteoporosis (OP) are becoming increasingly common in our ageing society. Bone is made up predominantly of a mineral phase (hydroxyapatite) and an organic phase (collagen). Collagen forms the main structural element of bone and understanding its behaviour in situ, specifically within diseased human bone, could lead to new understandings of these diseases.
This study investigates the intermolecular arrangement and packing of collagen molecules in trabecular bone using neutron diffraction. Neutron diffraction enables the collagen to be measured in the presence of mineral which otherwise obscures the protein if X-ray diffraction is used. The aim is to produce a theoretical model describing the molecular arrangement of collagen and how this depends on mineralisation and disease.
Femoral heads were obtained from consented patients who had undergone either a total or hemi- hip arthroplasty for OA and OP respectively. Coronal slices, approximately 2 mm thick, were obtained from near the midline of the femoral head and subjected to neutron diffraction performed at the Institut Laue-Langevin using the small momentum transfer diffractometer D16. A humidity chamber was used to control the humidity and temperature of the samples.
A model has been devised using diffraction theory based on collagen molecules in the equatorial plane having a short range two dimensional liquid-like order. From this a relationship between the recorded total intensity distribution and factors describing the collagen (scattering factor) and its organisation (form factor) have been derived. Preliminary results suggest that the molecular diameter appears smaller than expected, being approximately 1 nm, with a low packing fraction between 0.45-0.5. Statistical analysis of the sample data is being completed to compare the two disease types.

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CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF GLYCOSYLATION PROFILES IN MESENCHYMAL STROMAL CELLS

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N-Glycosylation is the addition of sugar residues to proteins in the ER and Golgi, which can alter their properties including their ability to bind to target proteins. Consequently, glycosylation has a major role in many cellular mechanisms including cell adhesion, signal transduction, and non-self-recognition, but its functional role in directing stem cell differentiation has been largely overlooked.
To identify how important glycosylation is during differentiation, we determined the glycan profiles of multipotent stromal cells/mesenchymal stem cells (MSCs) and then chemically altered the glycan profile and tested the effect on ability of MSCs to proliferate and differentiate.
Telomerase (hTERT)-immortalised clonal MSC lines were generated, to account for the broad glycan-heterogeneity often observed in cell cultures and to generate sufficient material for in-depth analysis of glycans. N-glycans were isolated from hTERT-MSCs using a novel, low-through put method with micro-centrifuge columns and PNGaseF. This method, followed by permethylation to stabilise glycans, allowed us to repeatedly profile samples using mass spectrometry (MALDI-TOF/TOF) and compare glycan abundance across different spectra, which has not been possible previously.
When grouped into glycan types, four MSC lines similarly had: 50% high mannose, 5% hybrid and 45% complex glycans. This is a lower abundance of high mannose glycans than previous reports, which identified 78% high mannose, 12% complex and 9% hybrid in 5 primary MSC samples. This difference is likely due to the permethylation included in our method, which stabilizes sialic acid residues, increasing complex glycan representation in our spectra providing a more accurate assessment.
Swainsonine, a mannosidase II inhibitor was used to disrupt the glycan synthesis pathway and caused a 50-fold increase in the proportion of hybrid type glycans in hTERT-MSCs without affecting proliferation rates or cell viability. Interestingly, culture of hTERT-MSCs with 10ug/ml swainsonine was found to enhance adipogenesis, even in the absence of adipogenic induction, as measured by Oil Red O lipid accumulation, compared to untreated controls.
These results suggest a strong role of glycosylation in stem cell differentiation with evidence that selected glycoprotein(s) are responsible for promoting adipogenesis. Differences in MSC glycosylation profiles may also act as suitable predictors of differentiation capacity.

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TEM IDENTIFICATION OF CONCENTRIC LAMELLAE IN ARTICULAR CALCIFIED CARTILAGE

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The structure, ultrastructure and function of hyaline articular cartilage (HAC) and subchondral bone (SCB) have been the subject of much investigation and the roles that these tissues play in the pathogenesis of osteoarthritis (OA) has been widely recognised. However much less attention has been focused on the intervening tissue, articular calcified cartilage (ACC). The ultrastructure of ACC is poorly described and it is not known to what extent it is involved in the initiation and progression of OA. We undertook a transmission electron microscopy (TEM) study of ACC in wild type (WT) mice and in mice with genetic or environmentally-induced OA to identify changes which may further our understanding of the role played by ACC in the early stages of OA.
Aged WT and OA mice were sacrificed, their knees fixed in either PBFS or glutaraldehyde for 24 hours, and decalcified for 7 days in EDTA. Samples underwent routine TEM processing, sectioning with an ultramicrotome, and post-staining with uranyl acetate and lead citrate.
In both WT and OA mice, we identified the appearance of concentric lamellae surrounding chondrocytes in ACC. These lamellar structures were associated not only with viable chondrocytes towards the calcification front, but also hypertrophic chondrocytes in the deeper zone of ACC. These concentric lamellae may be similar to those seen using SEM by Hirotani et al (1974) who proposed the existence of a lamellar system around chondrocytes in the deep matrix of the articular cartilage in patients with secondary OA, but other than that this is a novel finding in ACC of mice.
The identification of concentric lamellae in ACC is an important step in understanding how ACC may play a part in the early changes of OA. With the lamellae evident around hypertrophic chondrocytes, it seems areas of calcified cartilage are being replaced by bone. The resultant thinning of ACC due to its replacement by bone is characteristic of joints undergoing OA. The fact that the lamellae encircle hypertrophic chondrocytes, which release alkaline phosphates to calcify their surrounding matrix, shows that calcification and ossification in ACC may be an important step in the initiation of OA.

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IMPACT SIMULATION IN ORTHOPAEDICS - METHODOLOGY

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Impact is a critical loading scenario in orthopaedics. Impact can arise in vivo (eg stumbling), at implantation and from accidental loading. Impact loading is difficult to simulate and quantify as this is highly dependent on boundary conditions. Impact simulations should therefore be as close as possible to the situation simulated. A drop rig was developed to simulate a side fall.
A drop rig was designed. The literature was reviewed to determine the input parameters for a side fall. The set up was verified by testing porcine femurs and observing the resulting damage.
The impact test frame allowing drop heights up to 3m was manufactured. A speed sensor was attached to the base of the rig a few mms away from the impact position to verify the impact speed. The literature showed that a side fall onto a hard floor would be simulated by 140J of impact energy [Stephen 1995] with trochanteric tissue thickness of 24mm. The speed at impact was reported as 3.2m/s [Aya 1996]. With a drop weight of 20kg, the required drop height was 71cm.

Five porcine femurs were tested using this set up. The femurs were mounted onto a wood block so that the lateral side of the greater trochanter was directly under the impact. The femurs were placed in dry sand (approximately 5000cm3) to avoid any side displacement of the parts and to account for the surrounding body. A layer of 8mm of porcine tissue was placed at the position of impact to simulate the dampening expected from the trochanteric tissue in a worst case scenario. All five femurs split at the greater trochanter. The speed at impact was 3.5m/s.

A simple drop rig can be used to simulate impact loads for orthopaedic application. The test methodology should be developed for each impact conditions to be simulated, based on the literature. As well as impact energy and speed, special attention must be placed on boundary conditions, in particular at the point of impact. The method should be verified using as close a model to the conditions simulated as possible.

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HUMAN OSTEOBLASTS FROM DISTINCT BONE LOCATIONS EXHIBIT HIGHLY CONSERVED DIFFERENCES IN OSTEOGENIC AND PRO-ANGIOGENIC POTENTIAL

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Successful long-term, cementless fixation of human shoulder components in osteoporotic (OP) and osteoarthritic (OA) patients poses major challenges. The possibility that enhanced osseointegration may rely on both the region of bone targeted and its relationship with the vasculature remains unexplored.

We hypothesise that bone cells derived from subchondral (SC), cortical (C) and trabecular (Trb) bone regions exhibit differing osteogenic potential, which will be diminished in bones from OP patients. Primary osteoblasts from SC, Trb, C explants were obtained from OP (n=3) and OA (n=4) human patients undergoing shoulder arthroplasty and cell growth and gene/protein expression levels determined.

Cell proliferation studies consistently illustrated that osteoblasts from all sites in OA patients exhibited 20% ($p<0.01$) greater growth rates than from OP. Furthermore, osteoblasts from SC and C showed enhanced rates of proliferation, compared to Trb sites ($p<0.05$) in both OA and OP. Induction of osteogenic differentiation was found to promote greater increases in ALP activity and Osterix and Runx2 mRNA levels in Trb and SC, than in C bone osteoblasts ($p<0.05$) in OA patients; all OP sites exhibited significantly smaller increases in ALP activity ($p<0.05$). Vascular endothelial growth factor (VEGF) is an osteoblast-derived signal which couples osteogenesis and angiogenesis. We found that media conditioned by Trb osteoblasts from OA contain highest (21%) VEGF165/121 levels ($p<0.05$). Additionally, osteoblasts from all OA sites exhibited significantly higher VEGF mRNA/protein levels than OP ($p<0.05$). Our data indicated: i) that osteoblasts from all osteoporotic bone sites are likely to be compromised in their osteogenic potential, with limited growth, differentiation and VEGF production and ii) that osteoblasts from trabecular bone exhibit least proliferation, but greatest differentiation and pro-angiogenic potential, suggesting that they may provide for superior osseointegration. Together, these findings suggest that human osteoblasts with distinct positional origins exhibit divergent osteogenic potential.

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LACTOFERRIN INHIBITS GLUCOCORTICOID-INDUCED APOPTOSIS OF HUMAN ARTICULAR CARTILAGE CELLS IN VITRO

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The aim of this study was to test the anabolic effects of lactoferrin (LF) on human articular cartilage cells (HACs). HACs were isolated from osteoarthritic cartilage biopsies and cultured in low serum medium (DMEM/F12 supplemented with 1% fetal bovine serum [FBS]). The effect of recombinant human LF (rhLF) on dexamethasone-induced apoptosis was studied by assessing the viability and proliferation of HACs cultured for 2 days in low serum medium in the absence (control) or presence of dexamethasone (Dex) and / or rhLF using MTT and LIVE/DEAD assays, flow cytometry, qPCR, immunocytochemistry and confocal microscopy. The results showed that rhLF significantly enhanced HAC proliferation in low serum conditions, and partially rescued Dex-induced suppression of cell proliferation, possibly through up-regulation of the ERK signalling pathway. LF also significantly rescued Dex-induced apoptosis of HACs through inhibition of FAS, FASL and CASP3 expression. In conclusion, LF is an effective anabolic reagent on HACs from osteoarthritic cartilage tissue. This study will pave the way for further investigations of the potential clinical application of LF on osteoarthritis.

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NANO TO MICROSTRUCTURAL EFFECTS IN TENDON

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The nanometre scale is host to a range of interesting phenomena: it is at this scale that the basic language used for assembly, control and communication takes place in the biological environment. The major matrix components in the musculoskeletal system such as collagens, apatite crystals, elastin, proteoglycans, actin, and myosin are structured, and/or interact on this size scale. It is therefore an appropriate scale at which to study structure-function relationships in the matrix. Using a combination of scanning probe microscopy, nanomechanical testing, nonlinear optical microscopy and computational analysis, our group is investigating structure-function relationships in human musculoskeletal tissue at the tens of nanometre to hundreds of micron scales. Here we present findings on hamstring tendon.

We found distinct piezoelectric domains at the fibril-to-fibril level, and again at the tens of microns scale, create slipping planes within the tissue. Discrete 'clumps' of proteoglycan at the hundreds of nanometre scale, localised in areas of 20-30 microns diameter were also observed and mechanically tested. The distribution and properties of the components suggest a mechanism for the control of tendon deformation and recovery.

The piezoelectric property of the tissue aids the storage of elastic energy through a reorientation of the fibrils and the molecular dipole moments (polarisation) within them. Discrete piezoelectric domains, in which the sign of the piezoelectric (or second-order nonlinear optical susceptibility) tensor remains constant, stores energy and limits slipping within the domains. Between the domains, a slip plane is formed due to electrostatic repulsion, providing a mechanism to dissipate energy and limit damage.

The discrete distribution of proteoglycans indicates a capacity to further control deformation. Aggregated clumps of proteoglycan span multiple collagen fibrils, allowing finite sliding between them to dissipate energy while maintaining stiffness. It was also observed that the proteoglycans were stiffer than collagen, with a lower loss tangent, and exhibited less dissipation between the tip and sample, suggesting a greater capacity to store energy and potentially assist the recovery of crimp.

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ARE OSTEOARTHRITIS (OA) AND OBESITY ACCOMPLICES?

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Introduction:

Osteoarthritis (OA) is a multifactorial condition. Obesity is recognized predisposing factor and as the incidence of obesity increases in the population the prevalence of OA is also expected to increase. How

obesity contributes to the development of OA is not entirely clear but many concentrate on the increase mechanical loading in obese patients. Soluble factors, produced by adipose tissue adipokines, are thought to influence inflammatory, catabolic and anabolic pathways in joint tissues and thereby could potentially be involved in cartilage breakdown. The aim of this study was to determine if the adipokine (and adipokine receptors) levels were different in obese and lean patients with osteoarthritis.
Methods:
Consented 30 patients age from 49 to 78 took part in the study. Articular cartilage, infrapatellar fat pad and synovial tissue were collected from lean patients with BMI score of 25 or less and from obese patients with BMI score more than 35. Expression of adipokines and their receptors in cultured chondrocytes or unculture fat pad and synovium was assessed by semi-quantitative PCR.
Results:
The data show that for chondrocytes genes for adipocyte receptors such as ADIPOR1 and ADIPOR2 and CMKLR1 do not differ in expression. Chemerin was found to be higher in chondrocytes from obese OA patients compared to lean OA patients whilst expression of Visfatin and PPARG was down regulated in obese OA patient chondrocytes In the fat pad adiponectin expression was higher in obese OA patients.
Discussion:
These results indicate that the adipokine mileu varies in OA tissues depending on whether or not patients are obese. These are likely to influence the pathogenetic pathways that lead to OA in the different patient groups highlighting alternative therapeutic targets that could influence OA progression.

P 126

IDENTIFYING ARTHROPLASTY OUTCOME FROM PRIMARY CARE RESOURCE USE DATA

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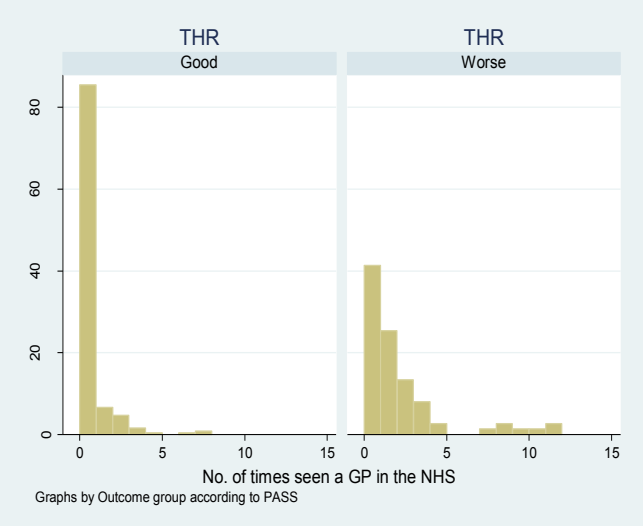
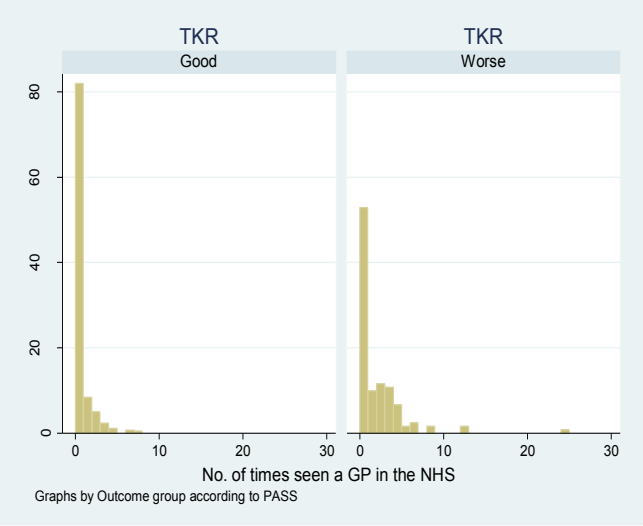
Background
Total hip (THR) and knee replacements (TKR) are two of the most commonly performed and successful elective operations in the UK. Whilst cost-effectiveness of both THR and TKR has been established, there is increasing need to expand research on surgical outcomes of dissatisfaction and chronic pain, particularly around the utilisation of health care resources. In the UK, the Clinical Practice Research Datalink (CPRD) is a large, comprehensive and representative database of primary care records but it lacks data on surgical outcomes.
Objectives
As part of the Study of Treatment After Replacement study (STAR) we aimed to estimate a model that identified surgical outcome groups based on their primary care resource use during the first 12 months after THR and TKR.
Methods
We used data from the first 314 recruited THR and 554 TKR patients in Clinical Outcomes in Arthroplasty Study (COASt) who completed the 12-month follow-up. Measures of resource use included number of visits to GP, hospital doctor, practice nurse, physiotherapist, alternative practitioners, and medication taken. Logistic regression modelling tested these as potential predictors of a good surgical outcome defined using a patient acceptable symptom state (PASS) for 12-month Oxford Hip (OHS) and Knee Score (OKS) related to satisfaction with surgery.
Results
Whilst 80% of THR (82% TKR) patients with a good outcome did not visit the GP because of their hip problems during the first year, for those with worse outcomes this dropped to 40% (53% TKR). The same pattern was observed with visits to hospital doctors, nurses and physiotherapists whereby worse outcome patients used noticeably more health care resources. The number of GP consultations, number of opioid drugs and taking non-opioids were statistically significant predictors of THR outcome. The area under the ROC curve was 0.80 suggesting good discriminatory ability. All explanatory resource use variables used in the model are available in CPRD.
Conclusions
Information on healthcare resource use in the CPRD can be used to identify patients with good surgical outcomes at 12-months following THR. Further work is needed to identify chronic pain patients after TKR but preliminary results point in the same promising direction.





P 127

ACTIVATED NATURAL KILLER (NK) CELLS AUGMENT OSTEOBLAST DIFFERENTIATION IN RHEUMATOID ARTHRITIS

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Rheumatoid arthritis is characterised by inflammation, cartilage destruction, bone erosion and systemic bone loss. However, even after resolution of inflammation bone is not repaired and the reason for this is unknown. One hypothesis is that inflammation leads to fundamental alterations in osteoblast progenitor cells (mesemchymal stem cells (MSCs) and fibroblasts). NK cells are an important cell type recruited to the inflamed joint constituting 20% of the lymphocytes in patients with established RA. These cells express an array of costimulatory molecules and produce significant quantities of cytokines and as such have major immunomodulatory potential. Despite this, little is known about their functional role; therefore the aim of the study is to investigate the role of NK cells on osteoblast differentiation. Using a model of cytokine activation to mimic the pro-inflammatory environment of the joint, Cytokine activated NK cells were shown to enhance osteoblast differentiation upon contact with MSCs, and the cytokine Oncostatin-M partially contributes to this. Interestingly,

soluble factors derived from NK-MSC interaction were insufficient to rescue the augmented ALP activity in proximal MSCs. It is our aim to identify the NK-expressed proteins involved in augmenting osteoblast differentiation as new therapeutic targets to promote bone repair.

P 128

PREVALENCE OF FRAGILITY FRACTURES IN PATIENTS WITH NORMAL RANGE OF T SCORE, OSTEOPENIA AND OSTEOPOROSIS

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Aim of this study was to compare prevalence of fragility fractures in three groups of patients based on their DEXA T score. We have performed 1958 scans on 1386 patients (94.5% female) in the period March 2010 - March 2013. Patents are either referred for DEXA scan by orthopaedic surgeons or by primary care.

573 (of 1386) patients reported 721 low intensity trauma associated fractures at the registration (first visit to our DEXA clinic). Of those 41.74 % (301/721) were wrist fractures, 19 % (137/721) were hip fractures and 10% were vertebral fractures. 20.6% of all fractures were reported with patients who had a T-Score in normal range,42.2% fractures were reported from osteopenic group of patients and 37.2% from osteoporotic group. 21.3% (122/573) patients within normal T-score range had reported low intensity trauma associated fractures, 44.1% (253/573) of osteopenic patients had fragility fracture and 34.6% (198/573) osteoporotic patients. There were 1.2 fractures per patients in group with normal range of T score, 1.2 in osteopenic group and 1.35 in group with osteoporosis.

Our analysis shows that there is no significant difference in fracture prevalence among groups with osteopenia and osteoporosis. Our analysis supports medical policy implemented in a number of world countries. Not only T Score can be used to measure probability of fracture and determine patients' eligibility to health treatment paid by the national health system. There is evidence to believe that change of medical practice in Serbia is required in order to enable more accurate calculation of probability of fracture and support better outcomes for patients. Further research is required to determine economical benefits of such policy but in the first instance we suggest that patients with T Score in osteopenic range can get prescribed (paid by national health service) medication. Current practice is that only those with lumber and/or hip T score less then or equal to - 2.5 are eligible.

P 129

TRABECULAR BONE SCORE IN TRAUMATOLOGY AND ORTHOPEDICS

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Introduction. Trabecular bone score (TBS) is a parameter of bone microarchitecture that is determined by the level analysis of DXA images. TBS is associated with fractures in the preliminary case-control and prospective studies.

The aim of this study was to assess the TBS role in the traumatology and orthopedics.

Materials and methods. We have examined 176 healthy women aged 40-79 years (mean age - 53.4±0.6 yrs) and 117 men aged 40-79 years (mean age - 59.8±0.9 yrs). Bone mineral density (BMD) of whole body, PA lumbar spine and proximal femur were measured by DXA method (Prodigy, GEHC Lunar, Madison, WI, USA) and PA spine TBS were assessed by TBS iNsight software package installed on the available DXA machine (Med-Imaps, Pessac, France).

Results. We have observed a significant decrease of TBS as a function of age (F=6.56; p=0.0003) whereas PA spine BMD was significantly increasing with age (F=4.04; p=0.008) in the examined women. This contradiction can be traced to the spinal osteoarthritis and degenerative diseases progressing with age in the elderly patients. TBS was significantly lower in women with duration of PMP over 4 yrs (p=0.003) in comparison with women without menopause; BMD of spine significantly decreased in women with duration of PMP over 7-9 yrs (p=0.02). So, the TBS can detect changes in the state of bone tissue at the earlier stage than BMD.

We have observed a significant decrease of TBS in men with ageing (F=2.44; p=0.05). Overall TBS values in men are lower than the age matched TBS values in women.

Conclusion. TBS is an independent parameter which has a potential diagnostic value of its own, without taking into account the BMD results. The study concerning patients with osteoporosis and fractures is underway.

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TRABECULAR BONE SCORE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Aim. To evaluate influence of age, duration of postmenopausal period (PMP) and duration of disease on trabecular bone score (TBS) and bone mineral density (BMD) of women with rheumatoid arthritis (RA).

Materials and methods. 129 women with RA aged 21-83 years were examined (age 52.4±12.7 yrs; height 162.6±6.4 cm; weight 68.5±13.8 kg; duration of disease 9.1±7.6 years). BMD of lumbar spine, proximal femur and total radius were measured using the DXA method (Prodigy, GEHC Lunar, Madison, WI, USA) and PA spine TBS was assessed by means of TBS iNsight software installed on our DXA machine (Med-Imaps, Pessac, France).

Results. We have observed a significant decrease of TBS in 50 year-old women with RA as compared to women aged 30-39 years (1.156±0.140 vs 1.318±0.155; p=0,001)/ The same was true of BMD of lumbar spine (0.994±0.245 vs 1.141±0.161 g/cm2; p=0.04), femur neck (0.716±0.245 vs 0.889±0.231 g/cm2; p=0.02), total radius (0.585±0.231 vs 0.722±0.141 g/cm2; p=0.04).

TBS is significantly lower in patients with a PMP duration of more than 3 years, as compared to women who were still menstruating (1.174±0.183vs 1.312±0.129; p=0.007). Femoral neck (FN) BMD significantly decreased when PMP duration was 5-10 years, as compared to women without menopause (0.682±0.254 vs 0.925±0.211 g/cm2; p=0.0004). A similar trend was observed in case of spine BMD (0.964±0.262 vs 1.133±0.164 g/cm2; p=0.001) and total radius, (0.526±0,221 vs 0.694±0.124 g/cm2; p=0,001) when the duration of PMP was more than 10 years.

Duration of disease did not influence TBS (with duration of the disease up to 3 years TBS was 1.214±0.166; 3-5 years - 1.221±0.162; 5-10 years - 1.255±0.162; over 10 years - 1.173±0.155; p=0,336). However, Total Radius BMD (0.600±0.178 vs 0.695±0.213 g/cm2; p=0.03) significantly decreased when RA lasted more than 3 years, spine (0.983±0.192 vs 1.115±0.181 g/cm2; p=0,008) and FN (0.654±0.224 vs 0.783±0.245 g/cm2; p=0,04) BMD when RA lasted more than 10 years, as compared to patients whose duration of RA did not exceed 3 years.

Conclusions. Age influences both TBS and BMD to the same extent, these parameters significantly decrease from 50 years onwards. TBS rapidly reacts to the changing hormonal status which is observed during menopause, and significantly declines after 3 years.

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ASSOCIATIONS BETWEEN VOLUMETRIC BONE MINERAL DENSITY AND DIETARY INTAKE IN SOUTH ASIAN AND CAUCASIAN WOMEN: PRELIMINARY ANALYSIS OF THE D2-D3 STUDY

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The evidence base confirming the necessity of sufficient vitamin D (in addition to other nutrients) in order to maintain skeletal health is well established. However, within the typical diet, few foods naturally contain vitamin D; thus the majority of the UK population rely on sun exposure during the spring/summer months for endogenous vitamin D production.

The aim of this study was to draw on data obtained from the D2-D3 Study (a double blind, vitamin D food fortification RCT) to evaluate

potential associations between skeletal health and dietary intake (particularly vitamin D) in South Asian (SA) and Caucasian (CA) women.
A cohort of 335 healthy women (CA n 245, SA n 90), mean age - 43.6±12.3 years; BMI - 24.1±3.8kg/m2 were recruited to the D2-D3 Study. At the baseline study visit, anthropometrics and a peripheral quantitative computed tomography (pQCT) scan of the radius were completed. Subjects completed a four-day record of their dietary intake. The pQCT data indicated that the CA women had significantly greater bone mass and area ($P<0.04$) at the distal radius. Yet at the same site, the SA women had significantly greater volumetric bone mineral density (vBMD, $P<0.009$). Dietary analysis indicated that for an average daily intake, the CA and SA women consumed similar amounts of energy and vitamin D; however the CA women consumed significantly greater quantities of potassium, calcium, magnesium, phosphate and alcohol ($P<0.05$). The comparison of tertiles of vitamin D intake with vBMD found no clear associations. Nor were there any correlations between vitamin D intake and vBMD. However for CA women, negative correlations were found between alcohol intake and bone mass, vBMD and trabecular density at the distal radius ($P<0.04$).
Further analysis of these diet/skeletal health associations are underway, particularly with respect to the higher vBMD detected in SA women despite a lower intake of the nutrients required for optimum skeletal health. Consideration of the impact of age, menopausal status and body composition on bone outcomes for both SA and CA women is also a priority.
The D2-D3 study is funded by the BBSRC DRINC programme (Grant No. BB/I006192/1)

P 132

IS SILVER A SAFE ANTIMICROBIAL IMPLANT COATING? INVESTIGATING HUMAN OSTEOBLAST VIABILITY ON SILVER-HYDROXYAPATITE ELECTRODEPOSITED IMPLANT COATINGS

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All implanted orthopaedic prostheses carry the risk of deep peri-implant infection. Currently around 1-2% of hip replacements become infected, resulting in considerable morbidity and high costs to the NHS. Silver's antimicrobial properties can be harnessed by incorporating it into an electrochemically deposited hydroxyapatite (HA) surface. These surfaces discharge silver in a large dose that tapers off to a controlled release. Because silver is cytotoxic to eukaryotic cells at high concentrations, this bolus" dose must be counteracted. It is theorised that preconditioning the coating in foetal calf serum (FCS) will ameliorate cytotoxicity to human osteoblast (HOb) cells.
This study aims to develop a reliable silver-HA co-deposition procedure using an electrochemical technique pioneered at our institute, and to determine if preconditioning these surfaces improves biocompatibility. The hypothesis is that silver-HA co-deposited surfaces which have been immersed in FCS for 24h will be less cytotoxic to HOb cells than untreated silver-HA co-deposited surfaces.
The effects of electrical current and stirring on coating thickness and silver content were investigated with scanning electron microscopy and energy dispersive X-ray spectroscopy.
In vitro experiments were carried out to assess cytotoxicity in HOb cells cultured on 4 different surfaces: bare titanium, electrodeposited HA, co-deposited HA + silver, and co-deposited HA + silver preconditioned in FCS. Cell viability, metabolism and morphology were assessed with live/dead staining, alamar blue assays and electron microscopy respectively.
It was found that stirring of the electrolyte improved coating uniformity and thickness. As predicted, the silver-impregnated coating was cytotoxic to HOb cells. Preconditioning the silver-HA co-deposited surfaces in FCS significantly improved cell viability; survival rates were similar to bare titanium controls. However, cell metabolism remained significantly diminished.
Preconditioning of silver-doped HA coatings with FCS can protect human osteoblasts from the most deleterious effects of silver cytotoxicity. However, more investigation must be conducted to determine if the observed metabolic decline is temporary, or if permanent cellular damage occurs. If silver impregnation can be made biocompatible, the co-deposition technique could be applied to a variety of uncemented orthopaedic implants."

P 133

OPTIMISATION OF FAST-FIELD CYCLING FOR DETECTION OF OSTEOARTHRITIC CARTILAGE

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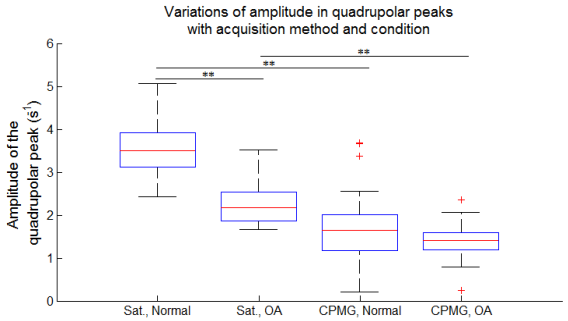
In contrast to conventional nuclear magnetic resonance (NMR) where a static magnetic field is applied to samples, the applied field in field-cycled (FFC) NMR is altered during the experiment. Measurements of relaxation rates (R1) as a function of applied field reveal interactions between water/protein protons and protein nitrogen nuclei (quadrupolar peaks; QP), the latter being proportional to protein concentration. In this study we examined the effect of two pulse sequences on QP amplitude of cartilage samples taken from patients with and without osteoarthritis (OA). This may have clinical significance in detection of early stage osteoarthritis in vivo.
Two experiments were designed using inversion recovery to assess QP amplitude. The first used saturation readout (500 points over 50 microseconds) whereas the second used CPMG readout (4096 points over 81 milliseconds). R1 was estimated by mono-exponential curve fitting.
After favourable ethical review was granted, patients were recruited prior to joint replacement surgery for hip fracture (n=12 for CPMG, 5 for saturation) or OA (n=15 for CPMG, 7 for saturation). The hip fracture patients did not have pre-existing OA clinically or radiographically. As routine protocol for the surgical procedure, the femoral head was removed and collected. Cartilage samples were subsequently harvested and analysed.
Significant differences in the QP amplitude were found between normal patients and those with OA from the saturation experiment (2.17 vs. 3.49; $p < 10\text{-}10$). However, CPMG experiments did not show significant difference between groups but the QP amplitude was significantly lower (1.61 vs. 1.41; $p <10\text{-}11$) (Figure 1).
The differences observed between saturation and CPMG are attributed to the short-lived NMR signal from cartilage proteins caused by the solid structure of their matrix. Fast measurements obtained directly after saturation pulses likely contain protein signal which varies with cartilage condition, whereas the CPMG sequence fails to measure fast-decaying protein signals. With CPMG, it is likely only water signals are recorded; this is uncorrelated to cartilage condition in our experiments.
Clinical experiments must therefore include rapid signal readout in order to take advantage of the large difference observed between OA and normal cartilage by FFC MRI.



P 134

CAN WE CHANGE THE WAY WE TRAIN? ADOPTION OF A NEW TECHNIQUE IN SURGICAL EDUCATION

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Introduction: Traditionally surgery has been an apprentice specialty with the trainee learning the art of surgery from his/her trainer during a period of attachment. Over the years there have been a lot of changes to

the practise of surgery in the UK and the rest of the world. With improved healthcare and rapid progress in the surgical field especially orthopaedics it is necesary to be well trained to avoid medico-legal implications in practise. We endeavoured to find the best possible way in which the trainee could master a surgucal skill in the least possible time.
Methods: Our unit is a tertiary level teaching hospital and there is a keen focus on registrar training. The senior surgeon (DN) regularly performs Anterior Cruciate Ligament (ACL) reconstruction in his practice. He uses a standardised operative technique. During the first two theatre sessions the trainee (SK) was taken through the procedure step by step. An operative guide and video was provided to SK to familiarise. During the next operating list SK vocalised each step to DN during surgery. Then SK was asked to be an extra assistant to help the scrub nurse. SK was confident of the procedure and was providing DN with the necessary instruments prior to being asked. The trainee was thinking ahead about the procedure and actively assisting.
Result: This novel method revealed a significant reduction of the tourniquet time by 30%.
Conclusion: This led to a greater satisfaction to the trainee and trainer. the trainee felt better trained with this method.

P 135

AWARENESS OF COMPARTMENT SYNDROME AMONG NURSING STAFF IN A TERTIARY LEVEL HOSPITAL

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Introduction: Compartment syndrome (CS) is an emergency condition that requires prompt recognition and treatment. Delay in prompt management may lead to irreversible complications, which may be loss of limb. Complications may arise as a result of delayed diagnosis, which included missed symptoms by nursing and junior medical staff or delay in treatment and inappropriate management. The aim of this study was to assess the understanding of CS among the nursing staff working on Orthopaedics Wards (OW) and Acute Surgical Ward (ASW).
Methods: A questionnaire was distributed among the nursing staff of the OW and ASW. This questionnaire assessed the knowledge of compartment syndrome including signs and symptoms and the appropriate management.
Results: 27 nurses completed this questionnaire, 17 from OW and 10 from ASW. 4% on the nursing staff were not aware of CS, 22% weren't aware of the signs and symptoms of CS and 89% weren't aware of the initial management of suspected CS. Only 11nursing staff had any training on identification and management of CS. Conclusion: Our data shows that although a majority of the nursing staff is aware of CS there still are areas of improvement. Regular workshops and teaching sessions especially to new staff members is necessary for the nursing

P 136

DOES FEMORAL TUNNEL POSITION IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION INFLUENCE THE OUTCOME?

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Background: There has been a lot of focus on the value of anatomic tunnel placement in ACL reconstruction, and the relative merits of single and double bundle grafts. Multiple cadaveric and animal studies have compared the effects of tunnel placement and graft type on knee biomechanics. The aim of this study was two-fold: establish the effectiveness of a single bundle ACL reconstruction technique in achieving anatomic femoral tunnel placement, and investigate a possible correlation between functional outcome and femoral tunnel position.
Methods: All patients underwent four strand hamstring graft single bundle ACL reconstruction, by a single surgeon, using antero-medial portal to drill the femoral tunnel as described by Pinczweski et al., which allowed more flexibility in placement of the femoral tunnel.
Results: Femoral tunnel position after ACL reconstruction in 45 patients was analysed by two independent doctors using the radiographic quadrant method as described by Bernard et al., and the mean values calculated. Forty-one of these patients undertook a KOOS questionnaire.
The mean ratio a was 26.57% and mean ratio b was 30.04% as compared to 24.8% (+/- 2.2%) and 28.5% (+/- 2.5%) respectively quoted by Bernard et al. Only twenty-three of these femoral tunnels were in the anatomic range. Analysis of forty-one KOOS surveys (23 anatomic, 18 non-anatomic) revealed no significant difference in total score or subscales between the anatomic and non-anatomic groups (p= >0.05).
Conclusion: Our study suggests that tunnel placement does vary between individual patients and is not necessarily as fixed as previously described.

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OUTCOME OF PROXIMAL FEMORAL NAIL ANTIROTATION (PFNA) IN THE MANAGEMENT OF FEMORAL FRACTURES. EXPERIENCE FROM A BUSY DISTRICT GENERAL HOSPITAL.

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Introduction: The incidence of proximal femur fractures has been on the rise recently. Subtrochanteric fractures or communited inter/pertrochanteric fractures present a difficult problem to an orthopaedic surgeon to stabilise the fracture and promote healing. Proximal Femoral Nail Antirotation (PFNA) from AO/Synthes has been in use for a few years and is known to provide good results. The aim of this project was to assess the outcome of PFNA in the treatment of hip fractures. Methods: Between November 2009 to November 2012 76 patients underwent PFNA for subtrochanteric of communited intertrochanteric fractures. These patients were identified from the local hip fracture database which is prospectively collected. The patients notes were reviewed. Results: The mean age of the patients was 80.11 years (range of 26.83 to 98.53). 27 were male and 49 female. Right hip was involved in 36 cases and left hip in 40. 9 patients required revision surgery due to failure of the primary surgery. 2 patients died due to other causes. 5 out of the 9 patient who underwent revision surgery had their primary surgery performed by a trainee under consultant supervision. The other 4 were performed by a consultant surgeon. There was a 11.8% failure of the PFNA in our series. 55% of these surgeries were performed by a trainee under consultant supervision. Conclusion: We conclude that PFNA is an excellent device to fix subtrochanteric or communited intertrochanteric fractures of thr proximal femur but on needs to be cautious and follow the correct operative technique to avoid failures.

P 138

MUSCLE PATTERNING IN PATIENTS WITH COMPLEX SHOUDER INSTABILITY

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Introduction
Those with atraumatic shoulder instability or Polar Type III under the Stanmore Classification[1], are a poorly understood patient group. The aim of the study was to use Electromyography (EMG) to investigate shoulder muscle activation during an arm elevation task based on activities of daily living.
Method
Five healthy controls and 5 patients with atraumatic shoulder instability were included. Surface electrodes were utilized to record the activity of 10 muscles: upper trapezius, serratus anterior; pectoralis major; biceps brachii; lattissimus dorsi, teres major, infraspinatus, anterior, middle, and posterior deltoid. Signals were recorded using a telemetry based EMG system during a reliable and accepted EMG testing protocol (based on the FIT-HaNSA functional assessment) which involved consecutive lifting of a weight from a low shelf to a high shelf (phase 1) and back (phase 2) [2] [3] .
32 patients/controls will have been recruited prior to the presentation of this work.

Results
Significantly greater activity (mean ± SEM) was seen in the latissimus dorsi during both phases of the movement protocol in the patient group: Phase 1 - 52.8% ± 9.1 vs 21.3% ± 6.7 (p-value 0.017); phase 2 - 52.8% ± 11.9 vs 18.9% ± 5.6 (p-value 0.044). No significant differences were identified in the other muscles of study.
Conclusion
Our study is the first EMG prospective study studying this patient group[4]. The study demonstrates that in those with atraumatic shoulder instability there is an over activation of Latissimus dorsi in both phases of a functional lifting activity.
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INFLUENCE OF GLUCOCORTICOIDS ON TRABECULAR BONE SCORE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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The aim of this study is to evaluate the influence of glucocorticoid therapy (GC) on the trabecular bone score (TBS), bone mineral density (BMD) and TBS dynamics during one year in patients with rheumatoid arthritis (RA).

Materials and methods. 134 examined women with RA (age 52.5±12.8 years; height 162.6±6.4 cm, weight 68.2±13.7 kg, duration of disease 9.1±7.5 years, duration of postmenopausal period 7.6±7.2 years) were divided into three groups: first group, G1, includes 37 patients who did not use GC, second group, G2 - 50 patients who used GC in a dose of more than 5 mg of prednisolone for more than 3 years, third one, G3 - 47 patients who took GC only at the exacerbated stage for less than 6 month. All the patients had been taking methotrexate as a basic treatment.

BMD of total body, PA lumbar spine, proximal femur and forearm were measured using the DXA method (Prodigy, GEHC Lunar, Madison, WI, USA) and PA spine TBS was assessed by means of TBS iNsight software package installed on our DXA machine (Med-Imaps, Pessac, France). Evaluation of TBS dynamics in the patients of G1 & G2 groups during the year was conducted on the background of ongoing therapy which included doses of GC (for the patients of second group) and/or without any osteotropic treatment.

Results. The 3 groups did not differ as to age, basic anthropometric parameters, duration of disease and duration of postmenopausal period in these groups.

TBS in G2 was significantly lower compared to G1 (TBSL1-L4: 1.147±0.168 vs 1.250±0.135; t=-3.07; p=0.003), and G3 compared to G1 (TBS L1-L4: 1.274±0.138; t=3.95; p=0.0002). However, there were no differences of BMD of PA spine and hip among groups. Only forearm BMD in the second group was significantly lower compared to the first one (0.583±0.176 g/cm2 vs 0.675±0.229 g/cm2; t=-2.18; p=0.032). Spine TBS decreased by 1.4% after one year for G1 and by 5.8% for G2.

Conclusion. For patients who are GC-users, TBS, but not BMD, reflects bone microarchitecture deterioration which is an indicator for those patients to of a higher vertebrae and non-vertebral risk of fracture. TBS is a determinant of bone state and must be monitored during the long-term treatment with GC.

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VITAMIN D DEFICIENCY IN PATIENTS WITH OSTEOPOROSIS

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Introduction. Vitamin D is important for calcium absorption and bone mineralization which is positively associated with bone mineral density. There is a direct relationship between BMD and fracture risk, with a decrease in bone strength and density associated with an increased incidence rate of fractures. Given the relationship between vitamin D and bone mineralization, optimal vitamin D status is essential for minimization of fracture risk.

The aim of study was to determined the frequency of vitamin D-deficiency and insufficiency in patients with osteoporosis.

Methods: There were examined 283 patients with systemic osteoporosis aged 40-94 years who were treated in department of age-related changes of musculoskeletal diseases D.F. Chebotarev Institute of gerontology. The average age of women - 65.26±0.60 yrs, men - 65.25±2.12 yrs. 25(OH)D and iPTH level was evaluated by electrochemiluminescence method (Elecsys 2010, Roche). Vitamin D deficiency was defined as level of 25(OH)D below 50 nmol/l, and vitamin D insufficiency as concentration of 25(OH)D of 50-75 nmol/l. Bone mineral density was measured by DXA Prodigy .

Results. The study shows that vitamin D deficiency was diagnosed in 80.7 % patients with systemic osteoporosis, insufficiency - in 11.5 % examined. Secondary hyperparathyroidism was diagnosed in 13.9 % cases. It was found significant correlations between 25(OH)D amount and bone mineral density at the level of Ward's zone (r=0.14, p<0.04), trochanter (r=0.18, p<0.01), proximal femur (r=0.16, p<0.02), lower extremities (r=0,14, p<0,04), forearm 33 % (r=0,13, p<0,05). 82.2 % patients with low-energy fractures has got vitamin D deficiency. In examined with vertebral fractures deficiency of vitamin D was registered in 86.5 %.

Conclusion. The revealed high frequency of vitamin D deficiency in patients with systemic osteoporosis make doctors to pay attention to 25(OH)D status and update the doses of vitamin D supplements in Ukraine.

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VOLUMETRIC BONE MINERAL DENSITY AND DIETARY PATTERNS ACROSS THREE AGE GROUPS OF CAUCASIAN AND SOUTH ASIAN WOMEN

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It is well established that low bone mass is the most important measurable determinant of osteoporotic fractures. Studies have shown an age-related decrease in bone mineral density (BMD) in both men and women. However in women rapid bone loss occurs predominantly during the menopausal transition. The objective of the study was to assess volumetric BMD (vBMD) and dietary intakes across three age groups (A:20-34yrs, B:35-49yrs, C:50-65yrs) of healthy women.

For this cross-sectional analysis, 335 women (n245 Caucasian, n90 South Asian) completed a baseline visit as part of The D2-D3 Study (a vitamin D food fortification trial). Data collected included anthropometrics, a four-day food diary and a peripheral quantitative computed tomography (pQCT) scan at the distal and mid-shaft sites of the radius.

Group A(n86), B(n134) and C(n115) had a mean age of 27.1±4.5yrs, 42.9±4.3yrs and 56.9±4.4yrs, and mean BMI of 23.1±4.3kg/m2, 24.0±3.4kg/m2 and 24.8±3.7kg/m2 respectively. BMI was significantly lower in group A than both B and C (p<0.001).

At the distal site, group C had significantly lower total vBMD than both A and B(C:299±50mg/cm3, A:321±48mg/cm3, B:323±46mg/cm3, p<0.001), and a lower trabecular vBMD than group A(C:176±34mg/cm3, A:188±31mg/cm3, p0.008). At the mid-shaft site, cortical vBMD was significantly different between all three groups, with density decreasing as age increased(A: 1131±55 mg/cm3, B:1130±147 mg/cm3, C:1107±107mg/cm3,p<0.016).

In group A, compared to both B and C, there was a significantly lower daily intake of protein(A:66±16g, B:74±18g, C:74±17g, p<0.007), potassium(A:2580±718mg, B:3039±929mg, C:3316±1106mg, p<0.001) and vitamin K(A35.8±57.2µg, B:50.9±70.4µg, C:50.8±47µg, p<0.010). Group A, compared to group C, also had a lower daily intake of magnesium(A:258±75mg, C:309±101mg, p0.001) and

phosphorus(A:1156±308mg, C:1293±263mg, p0.004). Alcohol intake was significantly higher in group C, than both A and B(C:13.2±12.0, A:5.6±10.1g, B:9.1±12.2g, p<0.003).
Despite higher intakes of nutrients known to have a positive role in skeletal health, the eldest age group had lower vBMD supporting the impact of age-related bone loss over the influence of dietary intake, on vBMD. Further research is underway examining the relationship between energy-adjusted nutrient intakes and bone health.
The D2-D3 Study is funded by the BBSRC DRINC Programme; LW is recipient of a BBSRC PhD Scholarship

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LOW BONE DENSITY AMONG FRACTURE NECK OF FEMUR PATIENTS: IS IT RELATED TO RENAL OSTEODISTROPHY? A SRI LANKAN STUDY.

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Introduction:
Fracture neck of femur (NOF) is a global orthopedic problem. Pathophysiology is related to the friality of body and fragility of bones which resulting disability. Associated osteoporosis is a major contributing factor for the fragility in neck of femur. Previous studies had elaborated the age related factors to the osteoporosis. Most of the NOF patients are suffering from renal insufficiency.
Objective:
To identify the probable relative risk of fracture neck of femur in renal impaired patients. Analyse the influence of renal function to the quality of bone. Identify the possibility of renal osteodystrophy related fragility fracture among the NOF patients.
Methodology:
Prospective cohort study among NOF patients (N=200) and compared with an age and sex matched control sample. Data were collected through a survey and direct observations. Variables studied are, Bone mineral density (BMD)of femur neck and lumbar spine, corrected serum calcium, serum phosphate, hemoglobin , blood urea, serum creatinine, serum albumin and serum protein of the individuals. Data were analyzed with logistic regression model with principal component analysis.
Results:
Logistic regression analysis revealed the most important variables to predict the relative risk of fracture are hemoglobin , blood urea and serum cretinine. Predicted probability of a fracture for hypoprotenemia 0.71, high blood urea 0.82, high serum creatinine 0.74. Blood urea , serum creatinine significantly higher (p-value<0.05)and serum albumin, haemoglobin, serum protein significantly (p-value<0.05) lower among the fractured group with compare to control group. BMD of lumbar spine and femur neck are significantly (p-value <0.05) lower among the fractured group with compare to control group.
Conclusion:
Above study shows the association of high blood urea, high serum creatinine and low hemoglobin with low bone mineral density in NOF patients. The relative risk of fracture is higher for renal insufficiency patients. No recognizable case control studies in English language literature. Studies in Nordic countries show higher incidence of renal impairment in NOF patients. Further studies are required to assess the relationship of renal osteodistrophy with NOF.

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DOES COLECALCIFEROL SUPPLEMENTATION HAVE ANY BENEFICIAL EFFECT ON THE BONE DISEASE IN HAEMODIALYSIS PATIENTS?

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Introduction and Aims: Vitamin D (Vit D) deficiency is prevalent in haemodialysis population. In addition to its classical effects on bone & mineral metabolism, its extra skeletal effects are increasingly reported in the literature. We conducted a prospective study of Colecalciferol supplementation in haemodialysis population with low Vit D levels. We used Renal Association (UK) guidance for vitamin D levels i.e. sufficient (>75 nmol/l), insufficient (37.5-75 nmol/l) and deficient (<37.5 nmol/l) groups.
Method: Serum 25 hydroxy Vit D [25 (OH) Vit D] levels were checked in prevalent haemodialysis patients. Patients with insufficient 25 (OH) Vit D levels were administered oral Colecalciferol 20, 000 units fortnightly while those with 25 (OH) Vit D deficiency received 20, 000 units weekly. Serum bone markers i.e. corrected calcium, Serum Phosphate, Parathyroid hormone (PTH) and C-Terminal telopeptide (CTx) levels were checked at baseline, 3 months and 14 months after supplementation.
Results: A total of 66 cases with inadequate Vit D levels participated in the study. Mean age of our population was 61 years (31-87yrs). Male to female ratio was 40:26. Forty six (70%) patients were deficient while 20 (30%) had insufficient Vit D levels. During the study period 14 patients died, 4 were transplanted, 1 changed dialysis modality and 1 moved to different area. Mean values of various bone markers during the study period were as follows,
Table 1

Serum bone markers	Pre Supplementation (n=66)	3 months Post supplementation (n=66)	14 months post Supplementation (n=46)
Mean Vit D levels (nmol/l)	32	111	105
Mean Serum Corrected Calcium (mmol/l)	2.35	2.41	2.31
Mean Serum Phosphate (mmol/l)	1.37	1.36	1.4
Mean Intact PTH (pmol/l)	27.7	30.0	35.9 (p=0.06)
Mean CTx (ugm/L)	1.91	1.86	2

Conclusion:
Colecalciferol supplementation improves Vit D levels. However, replacing Vit D has no significant effect on serum bone markers. Further studies are needed to explore the long term effects of Vit D supplementation in haemodialysis population.

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EVALUATING THE LONG TERM EFFECT OF BISPHOSPHONATE THERAPY

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In animal models bisphosphonates supress bone turnover causing unrepaired micro-crack accumulation. Anecdotally long term bisphosphonate therapy in humans has been associated with crumbling bone and a-typical fractures. As yet though no studies have demonstrated that bisphosphonates treatment is associated with micro-crack accumulation and reduced bone mechanical properties. This is in large part due to the inaccessibility of suitable human tissues and the technical difficulties associated with visualising and quantifying micro-cracks in 3D. This study aims to evaluate the long-term effect of bisphosphonate using state of the art Synchrotron micro-CT imaging at the Diamond Light Source (Didcot, UK). This paper presents preliminary research carried out using a variety of bench top micro-CT systems in preparation for the synchrotron scan time.
A sample of naive and bisphosphonate treated (>6 years) tissue samples have been collected from the femoral heads of osteoporotic fracture

patients. Cylinders (5.2mm in diameter and 12mm in height) were cored from the centre of femoral heads and micro-CT scanned at sub-micron voxel size. Finite element models were constructed based on the scans: two sets including and excluding micro-cracks respectively. In order to investigate whether micro-cracks weaken bone structure at a micro-scale the super element technique was used to model the stress and strain distribution around the cracks. Furthermore, the cores were experimentally tested to compare mechanical properties across the naive and treatment groups (as well as validate the finite elements models).
It appears as though bisphosphonates increase bone strength primarily via increasing volume fraction. Finite element analysis has been done on some samples with different average trabecular thicknesses. It is found that the strength is improved by 0.65% when the average trabecular thickness increased by 9.6%, which is from 143 μm to 156 μm. It shows that the increased trabecular thickness bought by bisphosphonate does not make big difference on bone strength.

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CHARACTERISATION OF ROUGHNESS AND SHAPE OF LONG BONE FRACTURES

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Fractures of the long bones are extremely common and most heal without complication. However, 10% ~ 15% do not heal successfully and can require a second surgical intervention. Despite the fact these fractures have been studied in the past, neither the mechanical behaviour of the fracture interface, or the complexity of its shape and/or properties have been fully characterised.
Therefore, the aim of this study was to develop a method for generating transverse fractures in vitro using porcine specimens and a computational code capable of characterising the shape, waveform and mechanical parameters of the fractures.
A drop-weight rig was developed that enabled transverse fractures to be artificially generated in porcine femoral specimens. Four paired porcine specimens were fractured, and then scanned using micro computer tomography (μCT) [XtremeCT, Scanco Medical]. The images generated where then imported to image processing software (Image J, http://imagej.nih.gov/ij/) and transformed into binary files. The first image of the stack of images for each specimen was then selected and a medial circumference was drawn using a skeletonise function. A code was then written (Matlab 2009b, Mathworks) to project each point on the circumference to the surface of the fracture to determine the height; these projections were then smoothed using a Gaussian Filter and different kernel sizes in order to observe the effect of the filter in the resultant smoothed curve. Finally, the smoothed curve was then subtracted from the original to enable different sizes of feature to be characterised.
Using small kernel sizes, the roughness of all the specimens was similar; however the larger shape of the fractures differed from one specimen to another. At a large scale, the fractures varied in amplitude from 6 mm to 16 mm.
From the results, it is clear that the fractures vary in overall shape but are more similar in terms of their roughness at a smaller length scale. These characteristics will now be used to develop computational models of different fracture types. The understanding of these factors will help researchers to improve the design of implants and aid the healing process.

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LOCALISATION OF SCLEROSTIN IN MUSCULOSKELETAL CELLS

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It is widely stated in the literature that the wnt signalling inhibitor sclerostin is found solely in osteocytes and that its sole function is to inhibit bone formation. However, occasionally publications have reported sclerostin in other musculoskeletal cells including chondrocytes, osteoblasts and osteoclasts. The objective of this study was to systematically investigate the production of sclerostin in human musculoskeletal tissues
Methods
Cartilage, bone and meniscal tissue were obtained from human donors under the appropriate ethics agreement and primary cell cultures of chondrocytes, osteoblasts, mesenchymal stem cells, tendon and meniscal cells prepared. Chondrocytic and osteoblastic cell lines were also investigated and also ovine mesenchymal stem cells . The presence of sclerostin and LRP5/6 was identified by Western blotting and immunohistochemsistry, using commercial antibodies Subcellular fractionation techniques were used to further analyse the distribution of sclerostin in the cells.
Results
Sclerostin protein was detected in all cells and tissues studied by Western blotting and immunohistochemistry with both antibodies. Subcellular fractionation of primary cells and cell lines indicated that sclerostin was detected predominantly in the nuclear fraction in addition to being present in the cytoplasm and membrane fractions. Immunohistochemistry demonstrated co-localisation of sclerostin and LRP5/6 in cells with punctate staining noted intracellularly, including in the nuclear and perinuclear region.
Conclusion
These results demonstrate that sclerostin appears to be ubiquitous in musculo-skeletal tissues and their derived cells. Our results also show co-localisation of sclerostin with LRP5/6 consistent with a recent report in myocytes of a clathrin-mediated endocytosis mechanism of internalisation of the sclerostin/LRP5/6 complex. In addition we have demonstrated previously unreported nuclear localisation of sclerostin.
Given that sclerostin has been considered by many authors to be an osteocyte-specific protein, the results presented in this study indicate that the control of sclerostin protein production is likely to be more complex than previously reported. Further studies are ongoing within our group to identify these roles and control mechanisms.

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KNEE MOMENTS OF ACL RECONSTRUCTED AND CONTROL PARTICIPANTS DURING SLOPE WALKING

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Prior injury to the knee, particularly anterior cruciate ligament (ACL) injury is known to predispose to premature osteoarthritis (OA). The study sought to explore the potential mechanism of this process by investigating changes in knee function during routine daily activities.
Twelve subjects who had undergone ACL reconstruction (ACLR) and 12 control volunteers with no history of knee trauma or injury were recruited into this study. Gait was assessed during normal, slow, uphill and downhill gait using a bespoke inclined walkway with an embedded Kistler Force plate (Kistler Type 9286B, Kistler Instrumented AG, Winterthur, Switzerland). A 10-camera Vicon motion capture system was used to capture the position of 23 markers attached to the subject (Vicon Motion Systems Ltd, Oxford, UK). The signals from the force plates were recorded at 1000Hz and synchronised with the motion capture data, recorded at 100Hz. Data was divided into gait cycles and time normalised. Joint angles and moments were calculated using a custom made model written in Body Builder software (Vicon Motion Systems Ltd, Oxford, UK).
No statistically significant difference was found in peak adduction moment between ACLR and control participants. Closer inspection revealed a discrepancy between the ACLR subjects. ACLR participants with menisci tear or collateral ligament damage (7 subjects) were found to have significantly higher adduction moment figure (0.33 +/- 0.12 Nm/kg m) compared to those participants with just ACLR figure (5 subjects, 0.1 +/- 0.057 Nm/kg m) during gait. The statistical test we used was One Way ANOVA, to give us $p = 0.042$. A similar trend was seen in all other activities (slow, uphill and downhill gait), however this did not reach statistical significance.
This work indicates that rather than an increased adduction moment those with ACLR had a reduced adductor moment thus questioning prior theories on OA development. In contrast those subjects who had sustained associated trauma to other key knee structures were observed to have an increased adduction moment. This suggests that perhaps it is the associated damage to the joint rather than the ACL injury itself that

contributes to the early degeneration frequently observed in this population.

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A RETROSPECTIVE STUDY COMPARING THE SURVIVORSHIP OF NON-INVASIVE AND MINIMALLY INVASIVE EXTENDIBLE BONE TUMOUR IMPLANTS

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This study investigated the survival of minimally-invasive (MI) and non-invasive (NI) extendible massive prostheses used in pediatric bone tumour surgery. Our hypothesis was that NI implants will have a higher survivorship due to a lower incidence of infection when compared with MI implants and that implants with a well integrated hydroxyapatite (HA) ingrowth collar will have lower aseptic loosening rates when compared with collars with no bony ingrowth.

188 patients received implants between 1994 and 2010. 63 MI's and 42 NI's fulfilled the inclusion criteria. Non-invasive implants (mean age 10.09yrs) were followed up to 7 years and MI's (mean age 11.67yrs) until 13 years post-implantation. A total of 20 implants were assessed radiographically (AP and ML). Length and width of radiolucent lines was measured and bone ingrowth within the HA collar quantified. Survival rates were estimated using Kaplan Meier curves and a Mantel-Cox Log Rank test used to compare survivorship curves. Data was analysed using the Spearman's Rank correlation where p values <0.05 were considered significant.

Survival at 1 year was 97.1±2.8% for NI implants and 96.7±2.3% for MI prostheses. After 7 years, the survival of NI prostheses was 40.4±14.4% and 45.0±7.9% in the MI group. At 13 years follow-up, the survival of MI prostheses had dropped to 36.0±10.2%. One (11%) NI implant was revised due to infection whereas 9 (35%) MI prostheses were revised. This was the major complication with MI prostheses. Full extension of the implant (and continued skeletal growth) accounted for 56% of NI and 15% of MI implant failures. This was the major complication affecting NI implants. None of the NI implants failed due to aseptic loosening however 23% MI prostheses failed for this reason. Radiographic assessment showed survival at 5 years was 87.5±11.7% for prostheses with integrated HA collars and 48.0±16.4% for prostheses with no bone growth within the collar ($p=0.017$).

Both MI and NI prostheses have similar survivorship rates at 7 years and analysis demonstrated that HA collars significantly reduced aseptic loosening. Minimally invasive prostheses are no longer used, however further work is needed to increase the growth potential of NI implants.

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MUTATED UBIQUITINATION REGION OF NEMO (IKKGAMMA) DOES NOT ABROGATE NFKAPPAB SIGNALING IN MYELOID CELLS OR ALTER BONE MASS IN GENETICALLY MODIFIED MICE

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Activation of the NFkappaB signalling pathway is crucial for normal osteoclast formation and bone resorption. Under unstimulated conditions, NFkappaB is retained in the cytoplasm bound to an inhibitory complex (IkappaB). Upon stimulation, such as RANKL binding of RANK, IkappaB kinase (IKK) phosphorylates IkappaB, targeting it for ubiquitination and proteasomal degradation.

The IKK complex consists of IKKalpha, IKKbeta, and IKKgamma, also known as NEMO. NEMO is a non-catalytic scaffold protein which facilitates assembly of IKK sub-units and interactions with the intracellular membrane. This is achieved through the formation of ubiquitinated structures attached to the NEMO protein, which in turn allow for downstream activation of NFkappaB signalling. Blocking NEMO binding with intracellular membranous proteins or other IKK sub-units abolishes NFkappaB activity.

To investigate the role NEMO ubiquitination plays in the regulation of NFkappB signalling, osteoclast formation, function and bone homeostasis, we studied genetically modified mice harbouring a point mutation (Lys392 to Arg392 (K392R)) that specifically disrupts K63-linked ubiquitination of NEMO at Lys392 (NEMO-KR mice). WT or NEMO-KR mice (male, 16 week old, n=8) were assessed using microCT imaging and histomorphometric analysis of decalcified long bones and undecalcified lumbar vertebrae, alongside osteoclast formation and activity assays (TRAP+ cell formation/dentine resorption) from primary mononuclear cells isolated from bone marrow. Downstream NFkappaB activity was determined by nuclear p65 levels and activation of target genes.

NEMO-KR mice demonstrated a non-significant 15% increase in bone volume/total volume (BV/TV)($p=0.17$) compared to WT, along with a non-significant 18% increase in bone mineral density ($p=0.11$). TRAP stained vertebral and long bone sections indicated no alteration in osteoclast number, corroborated by osteoclast formation and functional assays. Interestingly, downstream NFkappaB signalling did not seem to be affected by the point mutation in the known ubiquitin binding region of NEMO, with equal levels of nuclear p65 observed from WT and NEMO-KR cells.

These data suggests that whilst disruption of NEMO interactions with intracellular proteins and downstream signalling factors have been reported to block NFkappaB signalling, targeted disruption of crucial binding regions for ubiquitinated scaffold structures does not alter NFkappaB signalling in osteoclast precursor cells, osteoclast formation, function or bone remodelling in vivo.

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TUMOUR CELLS OUTSIDE OF THE LOCAL BONE MARROW CONTRIBUTE TO GENERALIZED BONE LOSS

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The production and sustained release of ectopic hormones by cancer cells, induce striking systemic effects in tissues distant to the tumour site. For example, untreated breast cancer patients develop decreased Bone Mineral Density (BMD) leading to increased vertebral fractures, compared to healthy individuals. Based on these clinical observations, we hypothesized that tumours at locations distant to bone may result in a generalized bone loss.

To investigate this we employed well-established murine tumour models of melanoma, breast cancer and multiple myeloma, to induce tumours at sites distant to the assessed region of bone. Bone volume was analysed by uCT scanning and histomorphometric analysis. Subcutaneous (s.c.) tumour growth was measured daily and tumour growth in bone quantified by GFP fluorescence and myeloma tumour burden determined by serum IgG2beta paraprotein levels. Urinary markers of bone resorption were also examined and dual-label fluorescent imaging used to assess bone formation rates (BFR).

Intravenous inoculation of 5TGM1 cells resulted in myeloma bone disease, with homing of myeloma cells to bone, osteolytic bone lesions, increased osteoclast number and decreased trabecular bone and BFR. In contrast, s.c. inoculation resulted in a time-dependent growth of a plasmacytoma, accompanied by a significant increase in serum IgG2 levels. Flow cytometric analysis detected no significant increase in GFP+ cells within the marrow, demonstrating that myeloma cells inoculated s.c. do not home to the bone marrow. Despite the absence of myeloma cells within the bone, uCT analysis demonstrated a 23% reduction in trabecular bone volume/total volume (BV/TV), and decreased BMD in plasmacytoma-bearing mice compared with non-tumour controls ($p<0.05$), accompanied by a significant increase in trabecular separation.

In addition, melanoma s.c. tumours resulted in a 20% decrease in BV/TV, decreased BMD and trabecular number, thickness and increased separation, while untreated bones in the intra-tibial MDA-MB-231 model showed a 47% decrease in BV/TV accompanied by decreased trabecular number, thickness and increased separation, along

with decreased BMD compared to non-tumour bearing animals ($p<0.01$).
Our data demonstrate that myeloma, melanoma and breast cancer cells induce generalized bone loss, providing evidence for the development of cancer-induced osteoporosis from tumours at sites distant to bone.

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INTER-ASSAY BIAS BETWEEN PARATHYROID HORMONE (PTH) ASSAYS AND ITS IMPLICATIONS FOR THE TREATMENT OF CHRONIC KIDNEY DISEASE MINERAL AND BONE DISORDERS (CKD-MBD) IN THE U.K.

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Introduction : Bioactive PTH is an 84 amino acid peptide that co-exists with various COOH-terminally truncated fragments (CTF's) in circulation. Hospital laboratories in the U.K. adopt different PTH quantification methods, which variably cross-react with these fragments. The clinical consequences of the resultant inter-assay bias are most discernible in CKD-BMD, as PTH informs often opposing and relatively contra-indicated treatment options. To minimise inter-assay bias, CKD-MBD treatment guidelines now use assay-specific reference-range multiples. However, manufacturer recommended ranges (MRR's) are very similar and therefore unreliable. Most healthcare professionals are unaware of this problem and the extent of inter-method bias in the U.K. has not been explored comprehensively. Methods: 1) Inter-assay bias between the five most commonly employed PTH assays in hospital laboratories was determined using U.K. National External Quality Assessment Service (UKNEQAS) data (2010-2013). 2) Mathematical modelling was applied to predict assay-specific (and therefore geographical) misclassification of CKD-MBD subtypes in renal dialysis patients treated at Brighton and Sussex University Hospitals (BSUH) during 2012 (n=1735). This was done using both MRR and Scottish renal registry (SRR) ranges. 3) Twenty UKNEQAS samples were analysed using fragment-specific ELISA's to determine influence on PTH concentrations reported by the nationally-adopted second-generations assays. 4) Calibration (against synthetic PTH) of all methods was determined using UKNEQAS data. Results: Inter-assay bias between nationally-adopted assays was 50% between the highest and lowest reporting methods. More than 300 patients in the BSUH population may have been classified and treated differently in geographical areas that adopt a different PTH assay. The overall misclassification between the most disparate methods reduced from 29% to 19% when SRR ranges were used. Both full-length PTH and CTF's were highly associated with nationally-adopted assays (r =0.95-0.99) ($p=<0.05$). However, a 2-fold difference in synthetic PTH recovery was seen between these methods. Discussion: Our data shows that significant inter-assay bias exists with PTH measurements throughout the U.K. Furthermore, this variability is likely result in the inappropriate and uneven treatment of CKD-MBD patients, and inaccurate audit of renal healthcare. Although the adoption of a commutable international standard will be beneficial, the multifactorial source of inter-assay bias must be considered. In the interim, we recommend the widespread adoption of SRR ranges and detail a novel way in which to cost-effectively assess inter-method variability. If validated, this approach could be used to provide a more definitive solution in the form of mathematical correction factors.

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DKK3 AS A MODULATOR OF CARTILAGE ACTIVITY

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Introduction: Our group has previously shown that Dkk3, a member of the Dkk family of Wnt antagonists, is upregulated in OA cartilage and synovium. Levels of Dkk3 in synovial fluid are increased in individuals with tricompartmental OA and after arthroscopy. The role of Dkk3 in cartilage or the factors regulating its expression are not currently understood.
Disruption of cell signalling pathways is important in controlling the development and progression of OA. Dkk3. a member of the Dkk family of Wnt antagonists, may therefore impact chondrocyte biology through interaction with the Wnt pathway. Dkk3 has also been found to influence TGFβ signalling in other cell systems.
Methods: Expression of Dkk3 was assessed in primary human articular chondrocytes (HAC) following cytokine trestment. Dkk3 expression was assessed following ex vivo injury of murine cartilage explants. The effect of Dkk3 on IL1/OSM-induced proteoglycan and collagen release from explants of bovine nasal (BNC)- and primary human-cartilage was assessed. Luciferase reporter assays and gene expression analysis were used to assess the impact of Dkk3 on Wnt and Smad signalling in human chondrocytes. Micromass HAC were treated with Wnt3a +/- Dkk3 and proteoglycan output assessed using alcian blue staining.
Results: Dkk3 expression was decreased in primary HAC following IL1/OSM treatment but increased by TNFa. Dkk3 expression was decreased following injury to murine explants. In BNC explants, IL1/OSM-induced proteoglycan release was inhibited by Dkk3. Dkk3 antagonized chondrocyte Wnt signalling and Wnt3a-induced reductions in proteoglycan production in micromass cultures. Interestingly, Dkk3 enhanced TGFβ signalling and antagonized Activin signalling.
Conclusions: Dkk3 expression is increased in OA and can be regulated injury and inflammatory cytokines. This suggests a balance of Dkk3 effects depending upon the biological stimuli within the cartilage. Dkk3 may act in a protective role in the presence of inflammatory cytokines as exemplified by its ability to inhibit matrix loss. The ability of Dkk3 to antagonize Wnt, enhance TGFβ and antagonize Activin signalling would have multiple effects on chondrocyte activity. These results imply that Dkk3 could influence multiple OA-relevant processes, protect cartilage from degradation and be important in cartilage development and homeostasis.

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NON-INVASIVE IN VIVO COLLECTION OF BIOCHEMICAL INFORMATION FROM OSTEOGENESIS IMPERFECTA HUMAN BONE; DEVELOPING METHODOLOGY FOR A CLINICAL INVESTIGATION

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Osteogenesis imperfecta (OI) is a genetic condition caused by a defect in collagen type I and is characterised by multiple fractures and skeletal deformities. The oim mouse is a model for human type III OI, and has reduced bone mineral crystallinity(1). Raman spectroscopy has been applied to measure differences between bone with different levels of mineralisation, and disease states(2), the development of Spatially offset Raman spectroscopy (SORS) allows chemical characterisation below the surface, e.g., bone through skin (3-6). SORS allows the examination of both the mineral and matrix phases of the bone.
The study aims to test the hypothesis: the Raman spectral signature of human OI bone will have reduced mineral crystallinity.
Human participants (controls=10, OI=10) were recruited into the clinical study (Ethical approval). The iSORS instrument (Cobalt Light Systems, Oxfordshire, UK) uses an 830nm laser, capped at 30mW per 3.5mm diameter aperture for use in vivo. The radius of the laser ring is changeable from 0-10mm, and spectra were collected at varying offsets for 1-3 mins. Spectra were baseline corrected to remove fluorescence and relative intensities of the mineral and collagen bands were calculated to yield the matrix ratios.
The bone spectrum was extracted for each participant, the averages compared and mineral crystallinity calculated(Fig.1).

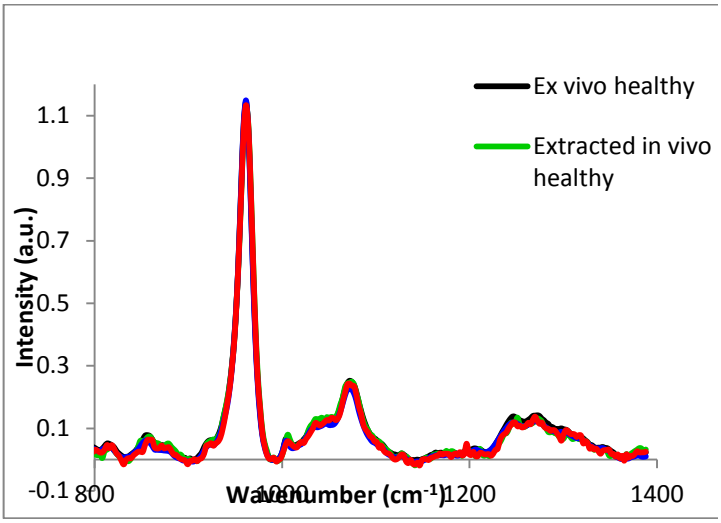

Fig. 1: Bone ex vivo and extracted in vivo Raman spectra from healthy and OI individuals.

Raman spectra of bone were acquired transcutaneously with optimised settings for spectra with a high signal to noise ratio. No difference in mineral crystallinity was found between the control and OI participants meaning the OIM mice are not good models for our patients, with less severe genetic mutations. The next stage of our work will be to explore differences between different types of human OI.

In conclusion, we demonstrate SORS has the capability of extracting biochemical signatures from bone, transcutaneously in vivo, providing an important tool to explore bone disorders.

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P 156

SEXUAL DIMORPHISM IN HUMAN TRABECULAR MICRO-ARCHITECTURE

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Introduction

As adults, women have thinner but more numerous trabeculae than men. Gradual thinning over time results in greater loss of trabeculae number in women whilst men due to their thicker trabeculae, do not experience the same loss in number. This is significant as a reduction in number results in a 2-5 times greater decrease in bone strength compared to a reduction in thickness. Although some evidence suggest that this difference is present in adolescence, the age at which men and women diverge is not clear. During gestation bone growth is thought to follow a predetermined trajectory (Cunningham and Black, 2009) although tissues can also adapt to loading (Skerry and Lanyon, 2009). In utero, males and females exhibit varied patterns of movement (i.e. punching, kicking etc.) with males displaying greater leg movements per minute. Thus sexual dimorphism might be expected to appear before birth. We investigated fetal bone development to determine whether males and females exhibit comparable trabecular micro-architecture during gestation and at term.

Methods

A sample of 25 humeri and femora from fetal skeletons aged between ~19-37 weeks was analysed. Limb bones were micro-CT scanned with a voxel size of 10 microns using an HMX-ST system (Nikon, UK). BoneJ and Quant3D were used to quantify trabecular micro-architecture in the proximal diaphysis. Six measures were collected: volume fraction; thickness; number; structure model index; connectivity density; degree of anisotropy.

Results

The trabecular architecture of males and females was comparable through gestation and at term. Trabecular bone volume fraction and thickness increased with age, trabecular number remained constant whilst anisotropy, connectivity and structure model index decreased (i.e. trabeculae became more highly ordered, less well connected and occupied greater bone density).

Discussion

The results from this study suggest that males and females could have similar pre-programmed developmental patterns and that trabecular structure only diverges between the sexes later in development. It is possible that sexual dimorphism arises during puberty when secondary sexual characteristics develop. One explanation for this divergence could be that females develop thinner and more numerous trabeculae to increase surface area for mineral homeostasis during pregnancy and lactation.

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AN ASSESSMENT OF FUNCTIONAL OUTCOMES AND PROPRIOCEPTION IN KNEE JOINT-SPARING ENDOPROSTHESES

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Background and Objectives: Where a bone tumour of the lower limb has not extended into the articular margin of the knee, a knee joint-sparing massive endoprosthesis may be used. These implants utilise extra-cortical plates that fix the implant to the remaining small segment of bone. In this study, we investigated proprioception, passive Range of Motion (RoM) and function in patients with knee joint-sparing endoprostheses and compared them to patients with conventional, joint sacrificing implants. Patients with Distal Femoral Replacements (DFR) and Proximal Tibial Replacements (PTR) were investigated.

Methods: Six patients with PTR, 5 with DFR, 3 with Joint-Sparing PTR (JS-PTR), 3 JS-DFR and 3 healthy subjects were evaluated. RoM for both healthy and operated limbs were measured. Limb function was evaluated using: (i) The Musculoskeletal Tumour Society Scoring system (MSTS), (ii) the Oxford Knee Score (OKS) and (iii) SF-36. Proprioception analysis involved measuring patient shank angle in relation to the horizontal plane. Patients were asked to repeat a specified movement 6 times and the error in repositioning of the shank measured. Mann-Whitney U tests were used for statistical analysis where p values < 0.05 were considered significant.

Results: A mean flexion angle of 1100 was measured in DFR patients with 1250 measured in JS-DFR given patients. No significant difference was found. A significantly higher mean flexion angle was measured in JS-PTR patients (1370) when compared with the PTR group (1030) ($p=0.037$). A mean SF-36 score of 60.15 was seen in JS-PTR patients when compared with 52.07 for the PTR given group ($p=0.055$). No significant differences were found when functional results were compared between patient groups. Proprioception analysis demonstrated no significant correlations when affected and non-affected legs in all groups were compared. No significant differences were measured in mean error when the joint-sparing, conventional or control groups were compared.

Conclusion: Knee joint-sacrificing implants remove the tibial tuberosity resulting in a poor extensor mechanism affecting limb function and movement. This study showed that joint-sparing endoprostheses provides enhanced RoM when compared with conventional joint sacrificing DFRs and PTRs ($p=0.037$). However, functional and proprioception analysis requires further investigation using increased patient numbers.

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A MID-TERM FOLLOW-UP OF KNEE JOINT-SPARING MASSIVE ENDOPROSTHESES

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Background and Objectives: Where a bone tumour of the lower limb has not extended into the articular margin of the knee, a knee joint-sparing massive endoprosthesis may be used. Instead of using the conventional intramedullary stem, these implants utilise extra-cortical plates that fix the implant to the remaining small segment of bone. This study reviewed the survivorship of knee joint-sparing massive prostheses implanted into patients between 1998 and 2007.

Materials and Methods: 51 patients (mean age 22-years) were included in this 11-year follow-up study. 54 patient-specific endoprostheses were implanted. These consisted of a total femoral replacement (n=2), distal femoral (n=27) and proximal tibial replacements (n=24). Implants were manufactured from Titanium alloy where the plateau and inside of the plate surface was hydroxyapatite (HA) coated. Fixation of the proximal stem in distal femoral implants and the distal stem in proximal tibial implants was either cemented or uncemented.

Results: Five patients died of complications unrelated to the implant, 4 were lost to follow-up and one patient's limb was amputated due to infection. Six implants were revised for non-implant related complications, including: infection, recurrence and a skip lesion. Two implants were partially revised at 3 and 61 months post operation, with plate fixation at the knee joint still in situ. One implant was revised after 77 months following a mid-stem fracture following a traumatic accident. Three tibial implants were revised for loosening of the extra-cortical plate fixation at 11, 20 and 22 months post-operation (12% failure rate). A survival probability of 0.93 and 0.68 at 10 years was estimated where revision of the implant due to failure of extra-cortical plates and revision for any reason was the end point respectively.

Conclusions: The overall risk of failure in joint-sparing endoprostheses is comparable with conventional joint-sacrificing distal femoral and proximal tibial replacements. Original concerns related to the use of joint-sparing implants, such as increased recurrence (n=1) and avascular necrosis (none reported) were unfounded.

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3D POSITIONING OF ACL ATTACHMENT SITES DURING FLEXION

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It is essential when performing anterior cruciate ligament (ACL) reconstruction that the replacement ligament has sufficient mechanical properties to function during all activities. In order to mechanically test a synthetic ligament and assess how well it will function, it is necessary to know how the device will be loaded both axially and in torsion for different activities.

We have developed a novel method by which the 3D movement of ACL attachment sites can be calculated from standard MRI data; which can then, in turn, be processed to estimate torsional and tensional loading data. The 3D shape of an individual patient knee was determined from standard MRI images using image segmentation techniques. The knee was then moved into position for each degree of flexion (ranging from -10deg to 140deg). The positions were known from published interventional MRI data which has recorded bone positions of ten healthy patients during flexion when weight bearing. Finally, the locations of the attachment sites throughout flexion were determined and the torsional and tensional loading within the synthetic ligament calculated. The ligament data were analysed assuming that the synthetic ligament was implanted at 90deg of flexion without pre-tension.

The results demonstrated significant twisting during hyperextension of the synthetic ligament (maximum 95.5deg), which would result in an approximate torsional force of 3.9N and a torque of 0.01Nm. This correlates with previous work by Zavatsky et al. which examined the mechanics behind ligament twisting [1]. Maximum ligament elongation (3.46mm) was found at 50deg of flexion, which would represent axial loading of 336N. For the calculations the synthetic ligament was assumed to be comparable to the native ACL, with a stiffness of 97Nmm-1[2], diameter 6mm and shear modulus 0.00172GPa.

This study highlights the importance of considering both ligament twist as well as elongation when testing synthetic ligaments for ACL reconstruction. Some in vivo trials of synthetic ACL replacement devices have found fibre wear to be an issue; it is possible that cyclic torsional loading tests could have predicted these problems.

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P 160

OSTEOARTHRITIS RATES POST DOUBLE BUNDLE ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION: THE STORY SO FAR

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Inadequate rotational control of the knee after standard Single Bundle (SB) Anterior Cruciate Ligament (ACL) reconstruction has been implicated in the development of osteoarthritis (OA). By reconstructing both the Posterior-Lateral and Anterior-Medial bundle, Double Bundle (DB) ACL reconstruction has proven to be superior to standard reconstruction in terms of both anterior-posterior and rotational stability of the knee in multiple functional and biomechanical studies. It has hence been postulated that DB reconstruction would show significantly lower rates of OA than standard SB repairs. We seek to test this theory by reviewing the results of trials specifically comparing OA rates in SB and DB reconstruction.

An English Language literature search on Medline was performed using the following terms: 'osteoarthritis', 'anterior cruciate ligament surgery' and 'double bundle reconstruction'. The search produced 1 Randomized Controlled Trial (RCT) of 90 patients and 1 Therapeutic Case Series of 50 patients.

Both trials failed to demonstrate any significant difference in OA rates between DB and SB reconstructions. The RCT evaluated patients 5 years post reconstruction according to Kellgren and Lawrence classification. When comparing OA rates in the medial, lateral and patellofemoral compartments, DB reconstructions did not show significant statistical difference compared with SB reconstructions. The case series evaluated patients at mean 4.4 years post surgery and no statistical difference was observed when OA was graded according to the Ahlback radiological criteria in the medial or lateral compartments.

While these early results seem to suggest that DB reconstruction has not reduced the rates of OA when compared to conventional reconstruction, they should be interpreted with caution. Firstly only 2 trials looking at this specific clinical question have been performed to date and the total number of subjects being compared is relatively small. Secondly, trials looking at the development of radiographic evident OA generally need to follow up their patients for a much longer time frame to obtain significant results. In addition, radiographic markers do not always correlate well with clinical symptoms of the patient, which have already been shown to be superior after DB reconstruction.

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ANALYSES OF ON ADMISSION BLOOD INVESTIGATIONS: FOR THE BETTER CLINICAL OUTCOME OF HIP FRACTURE PATIENTS IN ENGLAND.

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Objective:

To assess the value of analysis in on admission blood investigation for the outcome of fracture neck of femur English patients.

Methodology.

Prospective cohort observational study of 524 fracture neck of femur aged 60 or over and admitted to the Department of Orthopedics, Tameside General Hospital, Lancashire during year 2012. Pathological

fractures and fractures due to high velocity trauma excluded. 27.91% were male and female 72.08%. Univariate and multiple logistic regression analyses were performed to identify the relative contribution of the variables to mortality. Receiver operating characteristic (ROC) curves were used to identify optimal cut-off levels. Clinical data reviewed included age, gender, type of fracture, comorbid factors, on admission routine blood investigation and preop and post op duration of stay.

Results:

Patients were divided into 2 groups according to their survival after 90 days of injury. 160 patients (30.53%) were unable to survive more than 90 days. Survived group mean age(79yrs SD 13) and the deceased group mean age was 85yrs(SD 8.00) with pvalue<0.005. Significantly low haemoglobin (Mean11.50g/dl SD 1.80, p- value 0.0000), low haematocrit (mean 0.340 SD 0.05p-value <0.005), low packed cell volume (mean 3.73 SD 0.5, p-value <0.005) was observed in the deceased group. High blood urea (mean 8.89 mmol/L SD6.63, p-value < 0.005) and high serum creatinine(96.7 µmol/L ,SD 49.43, p-value 0.01) suggestive for on admission impaired renal function among the deceased group. White cell count, Serum electrolytes and EGFR (effective glomerular filtration rate) not had a significant impact on postop survival.

Conclusion:

Following fracture neck of femur, the 90 days mortality rate remain high around 30% in England over the last 20 yrs. Irrespective of the development and improvement in surgical care, orthogediatric care, rehabilitation services and social care it is remain static. Our study suggested low haemoglobin, dehydration and impaired renal function on admission had a direct impact on outcome of hip fracture patients. The evaluation of on admission blood investigation is prime important to improve the care of neck of femur fracture.

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IMPACT OF BIOLOGICAL AND STRUCTURAL FACTORS TO THE MID-TERM OUTCOME AND QUALITY OF LIFE IN FRACTURE NECK OF FEMUR PATIENTS. - A SRI LANKAN STUDY WITH COMPARE TO A COHORT IN ENGLAND.

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Introduction:

Fracture neck of femur (FNF) is a global orthopedic problem. Pathophysiology related to friality of body and fragility of bones. Incidence, mortality, morbidity depend on biological factors, living and health care standards of the individuals.

Objective:

Analyze the influence of biological, structural and physical variables to the post-operative mid-term morbidity and quality of life in fracture neck of femur patients; compare the results with global data.

Methodology:

Prospective cohort study (N=200). Variables studied are, Bone mineral density (BMD), Body Mass Index (BMI), corrected serum calcium, serum phosphate, hemoglobin level, blood urea, serum creatinine, level of social care, type of surgery, age and sex . The mid-term outcome and quality of life (QOL) is assessed according to the Harris Hip Score and EuroQOL. Data analysis done with logistic regression combined with stepwise forward selection procedure.

Results:

Serum albumin, hemoglobin, BMI and blood urea are the most significant biological factors to describe the severity of pain and mobility. Femur neck BMD is the structural component which describes the degree of pain and mobility. Physical variable analysis revealed age, surgery and levels, social care determine the level of mobility.

Conclusions:

Serum albumin, blood urea, hemoglobin, BMI, femur neck BMD, age and level of social care are the important variables that determines the quality of life in fracture neck of femur patients. Static factors are not correctable in acute patient care. Correction of serum albumin and hemoglobin, lowering of blood urea and improving the quality of postoperative social care will improve the outcome.

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OSTEOCYTES ARE INTIMATELY ASSOCIATED WITH THE REMODELLING OF MINERALISED CARTILAGE DURING OSTEOCHONDRAL REPAIR

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In osteochondral repair, producing the repaired cartilage/bone interface requires cartilage mineralization and its subsequent removal prior to bone deposition. However, in contrast to bone, mineralized cartilage does not contain cells that can remodel it and thus, little is known of the mechanisms involved in the control of this process. Recently, an in silico study hypothesized that osteocytes were the cell type most likely to be directing the remodeling of mineralised cartilage, in line with their spatial location, reported functions and known role in mechanotransduction. The aim of this study was to identify whether there was histological evidence for osteocytes remodeling mineralized cartilage in an in vivo model of osteochondral repair.

8mm diameter osteochondral defects were made in the medial femoral condyle of 24 mature sheep under the appropriate ethics. Defects were harvested after 6 months. Histological, histochemical and immunohistochemical analysis was performed on the defects including TRAP localization and immunolocalisation of sclerostin, Cathepsin K, MMP-13 and HTRA1.

In the region of active active healing, osteocytes were seen within the newly forming Haversian systems. Osteocytes were also noted in the region of active remodeling immediately adjacent to the newly formed cartilage and associated with cartilage islands. The osteocytic canaliculi were clearly seen to contact the mineralized cartilage. Additionally, it was observed that the cartilage edges had a scalloped edge appearance, similar in morphology to resorption fronts, with osteocytes present in the pits.

Sclerostin was absent in osteocytes immediately adjacent to the cartilage but sclerostin was present osteocytes outside the defect. Cathepsin K immunoreactivity was not detected within osteocytes in the normal bone. Within the osteochondral defect however, osteocytes adjacent to remodeling Haversian systems and the remodeling cartilage were positive for Cathepsin K. Osteocytes in this region were negative for MMP-13, HtrA1 and TRAP.

This study strongly supports the hypothesis, derived from in silico simulation models, that the osteocyte is intimately involved in the remodeling of mineralized cartilage at the cartilage/bone junction. In addition, we present preliminary evidence of a decrease in the wnt signaling inhibitor sclerostin and an elevation of Cathepsin K in osteocytes in the repair zone.

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UNDERSURFACE TIBIAL WEAR PATTERNS IN KNEE REPLACEMENT ASSESSED BY PROFILOMETRY

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Micromotion between the ultra-high molecular weight polyethylene (UHMWPE) component and metal tibial baseplate of total knee replacements (TKRs) is considered to be the causative mechanism for the generation of backside wear particulate debris.

Does tibial baseplate surface roughness influence the wear performance and surface topographical changes of the UHMWPE backside surface properties? This study utilized surface profilometry of retrieved TKRs to map topographical wear patterns of the UHMWPE undersurface component and reciprocal tibial baseplate surface.

Surface roughness (Sa) and skewness (Ssk) were measured in 16 topographical zones on both the UHMWPE component undersurface and the tibial baseplate of explanted knee joints using a non-contacting profilometer (1nm resolution). This allowed topographical surface analysis by mapping each zone to its corresponding articulating zone on the adjacent component. Fifteen complete retrievals were examined with two un-implanted prostheses for comparison.

Using a scatter-plot to compare tibial baseplate surface roughness with UHMWPE undersurface roughness for all articulating topographical zones on all explants, data points were found to be grouped into discrete clusters corresponding to individual retrievals.
This study found that surface properties of the tibial baseplate and backside polyethylene surfaces appear to change after implantation in a reciprocal wear process. This appears to be highly patient specific and as such may represent differences in in-vivo loading (patient demographics) and the behavioural response of the implant-bone construct.
The UHMPWE backside wear process is likely determined by a variety of both patient and implant factors that appear to combine to produce a unique wear fingerprint. Further work may generate a greater understanding of the influence of implant in-vivo longevity, patient characteristics (including morphology, activity and age) and biomechanics upon wear distribution.

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BONE AND JOINT PHYSIOLOGY EXPLORATION AND PATHOLOGY EXPLAINED

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Intraosseous pressure (IOP) in cancellous bone was measured experimentally and was found to vary considerably even when using a standardised technique. This has previously caused frustration with the technique for many years. However, superimposed on the variability in IOP there was a reliable underlying wave form with two distinct patterns. The patterns closely correspond to the arterial pulse wave and to the respiratory wave.
Systemic drug administration, physical joint and limb position changes, and joint loading also predictably affect the underlying IOP. Proximal arterial and venous occlusion have a direct and repeatable effect on IOP. When applied logically, combinations of these tests give useful information about cancellous subchondral bone circulation at the needle tip in vivo.
Triple simultaneous recordings in separate areas of cancellous bone appear to show that each bone behaves as a compartmentalised perfused sponge in a semiclosed system.
The Ficat saline stress test was used. The method is shown to damage local bone microcirculation. It does not appear to have a logical place in the functional exploration of bone or in assessing subchondral perfusion and vascularity. Aspiration may be more appropriate. There appears to be a subchondral microvascular 'bone blood pump' There is clinical, experimental and pathological evidence to support this fresh interpretation of bone physiology.
By applying these simple techniques in a controlled way a useful understanding of compartment syndromes and bone microcirculation, physiology and perfusion can be gained. IOP studies have been of limited value in the past but based on this interpretation can be of real value in understanding bone physiology and pathology.

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MODULATION OF OSTEOCYTOGENESIS IN 3D COLLAGEN GELS: EFFECTS OF FGF-2, IGF-1, VITAMIN K AND RETINOIC ACID

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Osteocytes differentiate from osteoblasts, are embedded in mineralised matrix and are critical regulators of bone remodelling. In vitro models are limited to cell lines in monolayer, which do not represent their 3D environment in vivo. We have shown that osteoblasts in 3D gels differentiate along the osteocytic pathway and that mineralising medium induces the expression of sclerostin, FGF-23, MEPE and PHEX by day 15. We have now investigated modulation of osteocytogenesis in 3D gels by FGF-2, IGF-1, vitamin k or retinoic acid (RA).
We maintained osteoblasts (MC-3T3) in 3D collagen gels (250 µl; 15 days) in alpha-MEM containing 10% FCS and +/- FGF-2 (10 ng/ml), IGF-1 (5 µg/ml), vitamin K (5 µg/ml) or RA (15 µg/ml). Cell number, viability and phenotype (confocal microscopy, IHC), IL-6, VEGF and FGF-23 secretion (ELISA), and gene expression (FGF-23, sclerostin, RANKL Cx43, DMP-1, E11; qRTPCR) were measured.
Cell number was decreased with RA (day 7, $p<0.001$) and vitamin K treatment, but was not modulated by FGF-2 and IGF-1. All factors decreased IL-6 (day 11 RA, FGF-2 both $p<0.05$), but increased VEGF secretion (day 15; RA $p<0.001$, IGF-1 $p<0.01$). FGF-23 mRNA was not detectable in untreated cells or at early time points with treatment, but was induced by RA, vitamin k, and FGF-2 from day 11 and by IGF-1 at day 15. FGF-23 secretion was detectable from day 11 with IGF-1, but was undetectable with any of the other factors at any time point. Sclerostin expression was undetectable other than with IGF-1 from day 3, and increased in expression with subsequent days (day 11, $p<0.05$). RANKL mRNA increased (e.g. 42- and 65-fold with IGF-1 and RA respectively on day 11, both $p<0.01$) with all treatments.
FGF-2, IGF-1, vitamin K and RA accelerated osteocytogenesis in this model, but IGF-1 induced both FGF-23 and sclerostin expression. All factors decreased IL-6 but increased VEGF secretion - both putatively mechano-regulated. Interestingly, RANKL expression was increased by all factors. Addition of factors such as IGF-1 to osteoblasts in 3D gels thus provides a novel and rapid method of generating osteocytes for future studies of osteocyte function, especially those relating to mechano-sensitivity.

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VDOP: OPTIMISING VITAMIN D STATUS IN OLDER PEOPLE. STUDY PROTOCOL FOR A RANDOMISED CONTROLLED TRIAL OF VITAMIN D SUPPLEMENTATION

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Vitamin D insufficiency is common in older people and may lead to secondary hyperparathyroidism, bone loss and impairment of muscle function. Vitamin D supplementation trials have yielded conflicting results with regard to decreasing rates of bone loss and falls and fractures; and, paradoxically, intermittent higher dose vitamin D supplementation has been associated with adverse events, including increasing rates of falls and fractures. Thus, optimal dose and frequency of vitamin D supplementation and target plasma 25-hydroxy vitamin D (25OHD) concentrations for skeletal health remain unclear. This trial will compare the effects of a range of doses of vitamin D, given each month for one year, on: BMD, plasma 25OHD, parathyroid hormone (PTH) and biochemical markers of bone turnover in older people to help in defining an adequate vitamin D intake and plasma 25OHD concentration to maintain musculoskeletal health.
Three-hundred and seventy-five men and women aged 70 years or older are recruited from general practices in the north of England, and randomised to a monthly oral dose of vitamin D supplementation (12,000IU, 24,000IU or 48,000IU) for one year starting in the winter or early spring. Hip BMD and anthropometry are measured at baseline and 12 months. Fasting plasma 25OHD, PTH and biochemical markers of bone turnover are collected at baseline and three monthly intervals to assess the dose-response and safety of supplementation. Questionnaire data include falls, fractures, quality of life, adverse events and outcomes, compliance, dietary calcium intake and sunshine exposure.
This is the first integrated vitamin D supplementation trial using a range of doses given at monthly intervals to assess BMD, plasma 25OHD, PTH and biochemical markers of bone turnover, and including safety measures, quality of life and physical performance. We aim to develop a set of biochemical markers that reflect the effect of vitamin D on musculoskeletal health and can be used in clinical practice. Our results will also aid in the interpretation of future larger studies investigating the effect of vitamin D supplementation on fracture risk and to evaluate the efficacy of the vitamin D supplementation.

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IN VITRO TRACKING OF CELL FATE PROVIDES EVIDENCE FOR ENDOTHELIAL CELL DEDIFFERENTIATION UPON DIRECT CONTACT WITH OSTEOBLASTS

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Inadequate revascularisation continues to impede the success of bone regeneration strategies and the addition of pro-angiogenic mediators such as Vascular Endothelial Growth Factor (VEGF) is often ineffective. Our previous studies showed that in transwell cocultures VEGF can regulate OB differentiation (Alkaline phosphatase; ALP) in an indirect fashion only when endothelial cells (ECs) are present. In contrast, direct contact/heterotypic culture of ECs and OBs increases ALP activity ($p<0.001$) but VEGF165 fails to exert any additive effect in this instance. We hypothesised that such increases in ALP activity coupled with a loss of sensitivity to VEGF was due to EC dedifferentiation in the presence of OBs. Further heterotypic cocultures were undertaken with mineralising MC3T3 osteoblast clones and human dermal microvascular endothelial cells (HMVEC) or GFP-HUVEC for up to 14 days. Protein expression was assessed by Western blotting for VEGF receptor 2 (VEGFR2) and alpha-smooth muscle actin (alpha-SMA) specific to EC and mesenchymal/OB populations respectively. Following coculture we saw a progressive reduction in VEGFR2 protein expression from 24-48 hrs which was undetectable after 7 days. In contrast alpha-SMA expression increased after 7 days of coculture. Studies with GFP-HUVEC showed ECs clearly present after 14 days in contact with OBs with phenotype often appearing elongated and fibroblastic. Double fluorescent labelling highlighted the presence of GFP -HUVEC positive for alpha-SMA following 14 days of coculture. Activin like kinase signalling is involved in driving an endothelial to mesenchymal transition in Fibrodysplasia Ossificans Progressiva [1] and preliminary studies utilising Activin like kinase-5 (ALK-5) inhibitors (SB431542 ; 10uM and A83-01 10uM) in EC:OB cocultures appeared to inhibit increases in alpha-SMA protein expression and maintain VEGFR2 expression levels and EC phenotype. Our findings could offer novel regimes for optimising bone regeneration by retaining endothelial cell phenotype and responsiveness to VEGF upon direct contact with mesenchymal cells via modulation of ALK5.

[1] Medici D et al (2010) Conversion of vascular endothelial cells into multipotent stem-like cells. Nature Medicine (16) 1400-1405

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TRANSIENT EXPOSURE TO ATP SENSITISES OSTEOBLASTS TO THE ACTION OF PTH FOR UP TO 9 HOURS

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ATP is released from osteoblastic cells in response to mechanical loading and acts on cells in the local environment via cell surface P2 receptors. Dual activation of P2 receptors and parathyroid hormone receptors (PTHR) on osteoblasts leads to a large induction in expression of bone genes including c-fos, RANKL and OPG. This synergy provides a mechanism whereby locally released extracellular nucleotides can sensitise cells to systemic PTH and thereby activate bone remodelling at discrete sites. To date the mechanism of this synergy has not been elucidated and it is not known whether the activation of the receptors has to be simultaneous or whether the cells have a memory of previous exposure. To test this hypothesis, we stimulated osteoblasts transiently with ATP and added PTH at time intervals up to 24 hrs. ATP (10μM) was added to SaOS-2 cells which had been stably transfected with a c-fos luciferase reporter, and grown to confluence in tissue culture wells. PTH (1nm) was subsequently added to different wells at hourly intervals. Simultaneous addition of PTH and ATP caused a 5 fold higher induction of c-fos than when PTH was added alone. Furthermore when PTH was added up to 3 hrs after ATP, there was an even greater increase than with simultaneous addition. The magnitude of the synergy declined with time but even 9 hrs after exposure to ATP, there was still a robust potentiation of the PTH effect on c-fos gene induction. The half-life of ATP in these cultures was less than 10 mins due to the action of ectonucleotidases, so that 1 hr after addition of ATP there would be insufficient nucleotide to continue activating the P2 receptors. Transient exposure to ATP was also found to potentiate subsequent PTH-induced stimulation of RANKL and inhibition of OPG expression. If addition of ATP, as evidence indicates, is mimicking at least part of the effect of mechanical strain on osteoblasts, these results imply that bone loading in vivo does not need to be coincident with PTH presence in the bone micro-environment to potentiate its action. This mechanism might contribute to cellular strain memory in bone remodelling.

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THE UNIGLIDE UNICOMPARTMENTAL KNEE REPLACEMENT: EARLY FUNCTIONAL OUTCOMES AND SURVIVORSHIP FROM AN INDEPENDENT CENTRE

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Background:

Unicompartmental knee replacement (UKR) is an accepted treatment for osteoarthritis isolated to one tibiofemoral compartment.

We present the early to mid-term results of a prospective consecutive series of 120 Uniglide UKRs with average follow-up 4 years from an independent centre.

Objectives are to identify:

1. The early functional outcomes; minimum 12 months
2. The survivorship
3. Errors in component positioning
4. Any complications associated with the prosthesis

Methods:

120 Uniglide UKRs were carried out in 107 patients between January 2006 and August 2012. All operations were performed by or under direct supervision of the senior author (PT).

Primary Outcomes: 1. Pre-and post-operative: American Knee Society Score, Oxford Knee Score, Western Ontario and McMaster Osteoarthritis Index. 2. Survivorship using revision as the endpoint.

Secondary Outcomes: 1.X-ray Error Score 2. Complications.

Results:

No cases were lost to follow-up. Two patients died of unrelated causes. Out of the 120 knees 6 were lateral and 114 were medial UKRs. There were 54 men and 53 women, 13 of whom had bilateral procedures. The mean follow-up time was 4.1 years (0.1 to 6.6 years). The mean age was 63 years (25 to 88 years). The differences in pre-operative and post-operative scores were statistically significant for each year of follow-up for each score. No significant change in score was found between annual follow-up scores. The survival at 6 years was 97%. There were three revisions: two from aseptic loosening of the tibial component and one secondary to disease progression. X-ray analysis identified the most common errors made as the femoral flexion/extension angle and tibial component overhang. The complications were 2 intra-operative: medial tibial plateau fracture and ACL partial avulsion; 2 early: post-operative haematoma and superficial wound infection and 5 late: 3 revisions to total knee replacement, formation of a neuroma at the distal end of the scar and a bearing dislocation.

Conclusion:

The Uniglide provides a significant improvement in early and mid-term knee functional outcome and satisfactory mid-term survivorship with an acceptable complication rate. Care should be taken when positioning the tibial component and aligning the femoral component.

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THE FPV PATELLOFEMORAL REPLACEMENT: MINIMUM 5 YEAR RESULTS FROM AN INDEPENDENT CENTRE

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Background:

This is the first independent study with mid-term follow-up of the FPV patellofemoral replacement. The purpose of this study was to assess the functional outcome and survivorship of this prosthesis.

Method:
This is a retrospective, multisurgeon case series. Between November 2004 and July 2008 three surgeons carried out 55 FPV patellofemoral joint replacements in 37 patients with isolated patellofemoral arthritis. The primary outcome measure was the Oxford Knee Score (OKS), secondary outcome measures were: Euroqol VAS general health score, survivorship, complications and radiological parameters- patellar height (Caton-Deschamps ratio), patellotrochlear index, notch position, patellar tilt, flexion/extension angle and assessment of bone interface. Survivorship and other analyses were performed including two-paired t test for OKS and Mann- Whitney for the Euroqol using a significance level of 0.05. Intra- and inter observer reliability analyses were also performed.
Results:
Three patients were lost to follow-up. In the first 5 years there were 5 revisions, giving a cumulative survival of 91%. The mean follow-up was 6.3 years (5.0 to 8.1). All the revisions were for disease progression. The median Oxford Knee Score was 31 (interquartile range 20.25 to 40.00). The Euroqol General health Score was 75. The main complication was disease progression which was identified radiologically in 18%. The most common radiological finding was radiolucency at the bone-cement interface of the patella.
Conclusion:
This study highlights the need for better patient selection and the importance of addressing underlying abnormal biomechanics. Our findings suggest that the FPV prosthesis gives satisfactory functional outcome in the medium term but is not comparable to the Avon which has been reported as 95.8 to 100% survivorship at 5 years. The FPV relies on meticulous positioning of the prosthesis due to the highly congruent design. Slight deviation from the required position may affect clinical outcomes.

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THE SURVIVAL RATE OF PATELLOFEMORAL REPLACEMENT AND TOTAL KNEE REPLACEMENT USED TO TREAT SEVERE ISOLATED PATELLOFEMORAL ARTHRITIS. A SYSTEMATIC REVIEW OF THE LITERATURE

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Background:
We systematically reviewed the literature to identify the survival rates and failure mechanisms of patellofemoral replacement (PFR) compared with total knee replacement (TKR) for the treatment of severe isolated patellofemoral arthritis.
Methods:
The National Library of Health search engine was used to search five electronic bibliographic databases, using the eligibility criteria, for literature published between the date of their inception and December 2012. A total of 17 articles met the inclusion criteria. The mean follow-up ranged from 1.6 to 17.0 years and the survival rate of the implants ranged from 75% to 100%.
Results:
The data heterogeneity excluded the option of assessment by meta-analysis. The mean
short-term (0-5 years) survival rate for first-generation PFRs was 94% (76-100), 94% (86-100) at mid-term (5-10 years) and 86% (75-100) in the long-term (greater than 10 years). For the second generation PFRs, three studies had a short-term survival rate of 100% whilst the remaining studies had a mean mid-term survival rate of 95% (88-100). The only TKR study in patients with isolated patellofemoral arthritis reported a mid-term survival rate of 93%. Of the total of 462 first-generation PFRs performed, 82 (18%) were revised. A significant number of revisions were secondary to malalignment and malpositioning. Forty-seven knees (10%) were revised to a total knee replacement, of which 39 (83%) had tibiofemoral disease progression. Of the 350 second generation PFRs, 19 knees (5%) were revised. All but one case was revised to TKR; 16 out of 18 (89%) were revised secondary to disease progression. Of the 31 knees treated with total knee arthroplasty, 2 (7%) required revision surgery.
Conclusion: Rigorous patient selection and understanding of patellofemoral biomechanics results in improvements in survival rates. The overall short to mid-term results for PFR are satisfactory. More PFR long-term data is required to compare with TKR and further support its use.

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EXTENSOR MECHANISM EFFICIENCY FOLLOWING PATELLOFEMORAL REPLACEMENT AND TOTAL KNEE REPLACEMENT: A CADAVERIC BIOMECHANICAL STUDY

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Background:
Weakness in the extensor mechanism after knee arthroplasty is not uncommon. The aim of this study was to determine whether geometrical differences between TKR and PFR resulted in dissimilar extensor moment efficiencies.
Method:
Extensor moment efficiency, patellofemoral joint reaction (PFJR) force, peak pressure and contact area were measured under four conditions: native knee, Zimmer PFR, cruciate-retaining- (CR) and posterior-stablising- (PS) TKR. Eight cadaveric knees were each mounted in a kinematic rig. Constant load was applied to the quadriceps muscles and ITB. Extensor moment efficiency was measured from 120 degrees to 0 degrees at 10 degree increments using a strain gauge; the other parameters were measured using Tekscan sensors. Analysis was performed using one-way ANOVA, post hoc paired t test and Pearsons correlation.
Results:
The key novel find was at 30 to 40 degrees knee flexion PFR produced the greatest extensor moment efficiency ($p < 0.008$) compared with native, CR- and PS-TKR. This suggests that PFR may be more efficient during the more functional range of motion; for instance during simple walking. The reverse occurred in deepest flexion although this is not of as great importance when performing activities of daily living.
The extensor mechanism efficiency negatively correlated with the PFJR force.
All the prostheses had significantly higher peak pressures compared with native. Significant reduction in PFR peak pressure corresponded with increased contact area.
Conclusion:
PFR had the highest extensor moment efficiency in early flexion possibly due to increased trochlear offset. This greater efficiency suggests PFR may be more beneficial during the functional range of motion. The claimed benefits of PS-TKR were not detected possibly due to tibial displacement.