ANZORS 21st Annual Scientific Meeting

Day 1: Clinical Education Centre, Auckland City Hospital, Park Road, Auckland

Day 2 & 3: Faculty of Medical and Health Sciences, University of Auckland, 85 Park Road, Auckland

October 2-4 2015
# ANZORS 21st Annual Scientific Meeting

## October 2-4 2015

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**LIST OF REGISTERED DELEGATES** 132
ANZORS 21st Annual Scientific Meeting

President’s Welcome

It is a great honour for us to host the 21st annual ANZORS (The Australian and New Zealand Orthopaedic Research Society) conference in Auckland from October 2-4, 2015. It is wonderful to see all you coming to the beautiful city of Auckland. We would like to welcome all of you; including distinguished guest speakers, presenters and attendants. It has been amazing to see that we have again received a record number of abstracts in 2015, signifying the right directions for ANZORS.

The beauty of this conference is that we all speak the same language in the orthopaedic realm. We hope that this meeting will provide you with a very friendly environment and a great opportunity to exchange scientific information, build collaboration networks, and promote both basic and clinical orthopedic research.

We thank all the participants for their contribution to the formation of this meeting, particularly the invited speakers, chairs, and committee members. Special recognition must go to Dr David Musson and Dr Justin Fernandez for being great local hosts, and to Dr Dominic Thewlis, Dr Egon Perilli and A/Prof Nathan Pavlos for their hard work in assembling this fantastic program and in financial issues.

We would like to express our deep gratitude and great appreciation for the generous support of the sponsors.

Finally, we wish all of you enjoy both the scientific programme as well as the beautiful season of Auckland. We hope that together, we can make our contribution to orthopedic research and translation. I wish this meeting a great success.

Prof Jiake Xu
President
The Australian and New Zealand Orthopaedic Research Society
School of Pathology and Laboratory Medicine
The University of Western Australia
Jiake.Xu@uwa.edu.au
ANZORS 21st Annual Scientific Meeting

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<tr>
<td><strong>President</strong></td>
<td>Head of Molecular Laboratory, School of Pathology and Laboratory Medicine, University of Western Australia</td>
</tr>
<tr>
<td>W/Prof Jiake Xu</td>
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<tr>
<td><strong>Secretary</strong></td>
<td>Senior Lecturer in Biomechanics, School of Health Sciences, University of South Australia</td>
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<tr>
<td>Dr Dominic Thewlis</td>
<td></td>
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<tr>
<td><strong>Treasurer</strong></td>
<td>Head of the Cellular Orthopaedics Laboratory, Centre for Orthopaedic Research, School of Surgery, University of Western Australia.</td>
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<td>A/Prof Nathan Pavlos</td>
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<tr>
<td><strong>2015 Host Organisers</strong></td>
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<tr>
<td>Dr David Musson</td>
<td>Research Fellow, School of Medicine, University of Auckland.</td>
</tr>
<tr>
<td>Dr Justin Fernandez</td>
<td>Senior Lecturer, Department of Engineering Science, University of Auckland</td>
</tr>
<tr>
<td><strong>Immediate Past President</strong></td>
<td></td>
</tr>
<tr>
<td>Prof Hala Zreiqat</td>
<td>NHMRC Senior Research Fellow and Head of the Tissue Engineering and Biomaterials Research Unit, Faculty of Engineering, University of Sydney</td>
</tr>
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</table>
Organising Committee

Dr David Musson
Dr Justin Fernandez
Dr Sue McGlashan
Dr Dominic Thewlis
A/Prof Nathan Pavlos
Dr Egon Perilli
W/Prof Jiake Xu
Prof Hala Zreiqat
Xiaoming Wang
Dr Geoffrey Hansfield
Dr Ju Zhang
Christopher Miller
Marco Schneider
Wilson Fok
Andi Liu
Helen Liley
Shasha Yeung
Alex Swee
Mousa Kazemi
Scientific Committee
(alphabetical surname order)

Dr John Arnold

A/Prof Thor Besier

Dr Tania Crotti

Dr Justin Fernandez

Prof David Findlay

Dr Claire Jones

Dr Pazit Levinger

Dr Zufu Lu

Dr Saulo Martelli

A/Prof Nathan Pavlos

Dr Egon Perilli

A/Prof Peter Pivonka

Dr Melissa Ryan

A/Prof Bogdan Solomon

Dr Dominic Thewlis

Prof Cory Xian

Prof Jiake Xu

Prof Hala Zreiqat
ANZORS 21st Annual Scientific Meeting

Travel Grant Recipients
(alphabetical surname order)

ANZORS is proud to support its early career researchers. This year we have awarded 25 travel grants. This represents a significant reinvestment of our funds to support the dissemination of quality orthopaedic research.

<table>
<thead>
<tr>
<th>Surname</th>
<th>First name</th>
<th>Institution</th>
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<tbody>
<tr>
<td>Ab Lazid</td>
<td>Rosidah</td>
<td>Flinders University</td>
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<tr>
<td>Algate</td>
<td>Kent</td>
<td>University of Adelaide</td>
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<tr>
<td>Beck</td>
<td>Aswin</td>
<td>University of Western Australia</td>
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<tr>
<td>Blanchard</td>
<td>Romane</td>
<td>University of Melbourne</td>
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<tr>
<td>Chan</td>
<td>Audrey</td>
<td>University of Western Australia</td>
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<td>Coates</td>
<td>Gino</td>
<td>University Of Melbourne</td>
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<tr>
<td>Devanaboyina</td>
<td>Pavan Teja</td>
<td>Griffith University</td>
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<tr>
<td>Fraysse</td>
<td>Francois</td>
<td>University of South Australia</td>
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<td>Hua</td>
<td>Martin</td>
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<td>Huang</td>
<td>Rong</td>
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<tr>
<td>Lamberto</td>
<td>Giuliano</td>
<td>The University of Sheffield</td>
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<tr>
<td>Lerebours</td>
<td>Chloe</td>
<td>Monash University</td>
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<tr>
<td>Leta</td>
<td>Tesfaye</td>
<td>Norwegian Arthroplasty Register</td>
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<tr>
<td>Lu</td>
<td>ZuFu</td>
<td>The University of Sydney</td>
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<td>Malekipour</td>
<td>Fatemeh</td>
<td>The University of Melbourne</td>
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<tr>
<td>Martelli</td>
<td>Saulo</td>
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<tr>
<td>Mekhileri</td>
<td>Naveen</td>
<td>University of Otago</td>
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<tr>
<td>No</td>
<td>Young Jung</td>
<td>University of Sydney</td>
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<tr>
<td>Roberts</td>
<td>Bryant</td>
<td>Flinders University</td>
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<td>Robertson</td>
<td>William</td>
<td>The University of Adelaide</td>
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<tr>
<td>Tickner</td>
<td>Jennifer</td>
<td>University of Western Australia</td>
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<tr>
<td>Timmi</td>
<td>Alessandro</td>
<td>The University of Melbourne</td>
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<tr>
<td>Trichilo</td>
<td>Silvia</td>
<td>The University of Melbourne</td>
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<tr>
<td>Wang</td>
<td>Xin</td>
<td>Queensland University of Technology</td>
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<td>Zhou</td>
<td>Yinghong</td>
<td>Queensland University of Technology</td>
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</table>
Dr Lin Han

Lin Han obtained his B.E. degree at Tsinghua University in P. R. China, and his Ph.D. degree at the Massachusetts Institute of Technology. His Ph.D. thesis focused on the molecular, cellular and tissue nanomechanics of cartilage. He later worked as a post-doctoral associate in the Department of Materials Science and Engineering and the Center for Biomedical Engineering at MIT, where he continued the exploration of nanostructure and nanomechanics of soft and hard biological tissues. During this period, he developed an array of unique nanomechanical tools to uncover the molecular origins of cartilage development, function and disease. In Nov. 2012, he joined the School of Biomedical Engineering, Science and Health Systems at Drexel University as an assistant professor. His current research seeks inspiration from the nanoscale structure-mechanics of synovial tissues to their development and maturation, aging and OA progression, with a special focus on hyaline articular cartilage and fibrocartilage (meniscus and temporomandibular joint). The ultimate goal is to provide a fundamental knowledge basis for the application of disease diagnostics, tissue regeneration and bio-inspired material design.

Dr Gethin Thomas

Dr Gethin Thomas is Head of the Skeletal Biology Group at the University of Queensland Diamantina Institute (UQDI) in Brisbane, Australia.

He undertook his undergraduate and PhD studies in the Department of Biology at the University of Birmingham in the UK, graduating with a PhD in Bone Cell Biology. He then undertook post-doctoral studies at the University of California in San Diego for two years followed by five years in the Bone and Mineral Research Program at the Garvan Institute. Dr Thomas then moved to industry spending five years with a biotechnology company in Montréal leading a project characterising novel bone genes. This resulted in the identification and characterisation of two novel bone genes, Ostn and Bril, with Ostn being patented and targeted for drug development and Bril being identified as the gene mutated in osteogenesis imperfecta type V. In 2005, Dr Thomas returned to academia in Australia to work with Professor Matthew Brown in the Human Genetics Group, focusing on the functional characterisation of genes identified by genetic screens in bone and joint disease.

His current research interests focus on the functional validation of candidate genes in ankylosing spondylitis (AS), a form of arthritis in which inflammation drives excessive bone formation. These studies are undertaken using gene expression profiling and molecular and cellular studies in both clinical samples and mouse models. He has recently completed the first large-scale RNAseq study in AS. Utilising the proteoglycan-induced spondylitis (PGISp) mouse model of AS he has characterised the molecular and histological changes occurring during disease progression to identify potential therapeutic approaches.

Since Oct 2011, he has also been the Deputy Director of Education at UQDI.
**Prof Hala Zreiqat**

Professor Zreiqat is recognised internationally as a leading authority in orthopaedic biomaterials research and is regularly invited to speak at national and international conferences. She has published 92 research papers, 8 book chapters and 7 review articles and is primary editor of A Tissue Regeneration Approach to Bone & Cartilage Repair (Springer, 2015). Professor Zreiqat has obtained >$8.2M in competitive funding. Her pioneering development of innovative biomaterials for tissue regeneration has led to one awarded (US) and 6 provisional patents, 5 as a lead inventor, and several collaborations with international industry partners.

She received 3 honorary professorial appointments at universities in the USA, China, Thailand and Lebanon. She is the immediate Past President, ANZORS; Founder & Chair, Alliance for Design & Application in Tissue Engineering (partners include Harvard, Columbia, Stanford, Tufts, Hong Kong); Co-founder: Sydney Bone Group (2002 onwards). Awards: The Rebecca Cooper Research Foundation PhD Scholarship (2016-18), John & Eileen Haddon Award (2015), Leopold Dintenfass Memorial Award (2012) for Excellence in Research, and Australia-Harvard Fellowship (2013).

**Prof Gary Hooper**

Professor Gary Hooper is Head of Department, Orthopaedic Surgery and Musculoskeletal Medicine, Christchurch School of Medicine and Health Sciences, University of Otago Christchurch. He is a past president of the New Zealand Orthopaedic Association. He has been a consultant Orthopaedic Surgeon with the Canterbury District Health Board since 1985. His main areas of interest include knee injuries and adult reconstructive hip and knee surgery. He is active in undergraduate and postgraduate education and heads a research group specialising in bioengineering of articular cartilage, implant development, joint replacement outcomes, rehabilitation following musculoskeletal disorders and fracture healing. The New Zealand Joint Registry and New Zealand ACL registry work within his department.

**A/Prof Thor Besier**

Thor is an Associate Professor at the Auckland Bioengineering Institute and has a joint appointment with the Department of Engineering Science at the University of Auckland. He completed his PhD in musculoskeletal biomechanics at The University of Western Australia in 2000 and was a postdoctoral fellow in the Bioengineering Department at Stanford University from 2003 to 2006. Thor established Stanford's Human Performance Laboratory as the Director of Research and was a faculty member in the Department of Orthopaedics at Stanford from 2006 to 2010, before returning home to New Zealand in 2011.

Thor’s research combines medical imaging with computational modelling to understand mechanisms of musculoskeletal injury and disease. In particular, he is interested in the mechanical aetiology of patellofemoral pain and osteoarthritis and novel technologies to diagnose and treat these disorders. He has published more than 75 scientific articles on these topics and currently receives funding from the NZ Marsden Fund, the US Food & Drug Administration (FDA), the Australian Research Council and the Australian National Health & Medical Research Council.
Venue

Day 1: Clinical Education Centre, Auckland City Hospital, Park Road, Auckland

Fisher & Paykel Healthcare
Clinical Education Centre
Whare Ako Rongoa

From the carpark:
Walk along the walk way to level 1 of the main building—follow the blue lines.
Take the public lifts to level 5. Turn right and follow signs to the Clinical Education Centre (CEC).

From the main entrance:
Walk through the main entrance to the new hospital.
Take the escalator to level 5 and turn left. CEC entrance is a few steps ahead.
Day 2 & 3: Faculty of Medical and Health Sciences, University of Auckland, 85 Park Road, Auckland
Program
Day 1 (Friday October 2) - Clinical Education Centre, Auckland City Hospital, Park Road, Auckland

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<tr>
<td>08:30-08:45</td>
<td>President’s welcome</td>
<td>W/Prof Jiake Xu</td>
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<tr>
<td>08:45-09:25</td>
<td>Session chair: W/Prof Jiake Xu</td>
<td>Keynote: Dr Lin Han</td>
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<tr>
<td></td>
<td>“Uncovering Murine Knee Joint Function and Pathogenesis via Nanomechanics”</td>
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<tr>
<td>09:30-10:30</td>
<td>Podium 1</td>
<td>First name</td>
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<td></td>
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<td>Jennifer</td>
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<td>Taryn</td>
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<td>Lennex</td>
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<td></td>
<td></td>
<td>Samuel</td>
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<td>10:50-11:35</td>
<td>ANZORS-China 1</td>
<td>First name</td>
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<td>Ling</td>
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<td>Ming Hao</td>
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<td>11:35-12:35</td>
<td>Oral posters 1</td>
<td>First name</td>
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<td></td>
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<td>Martin</td>
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<td>Alex</td>
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Lunch & poster viewing (poster viewing will take place between 12:45-13:30)
<table>
<thead>
<tr>
<th>Time</th>
<th>First name</th>
<th>Surname</th>
<th>Abstract title</th>
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<tbody>
<tr>
<td>13:45-14:25</td>
<td></td>
<td></td>
<td><strong>Keynote: Dr Gethin Thomas</strong></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>“Inflammatory bone formation – associative or causative”</td>
</tr>
<tr>
<td>14:25-15:25</td>
<td>Dale</td>
<td>Robinson</td>
<td>The capacity of isotropic hyperelastic material to describe contact of human articular cartilage</td>
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<tr>
<td></td>
<td>Francois</td>
<td>Fraysse</td>
<td>Peak Loading During Walking is Not Associated With Fracture Migration Following Tibial Plateau Fracture: A Case Series of Nine Patients</td>
</tr>
<tr>
<td></td>
<td>Chloe</td>
<td>Lerebours</td>
<td>A multiscale mechanobiological model of bone remodeling predicts site-specific bone loss in the femur during osteoporosis</td>
</tr>
<tr>
<td></td>
<td>Mark</td>
<td>Williams</td>
<td>Sensitivity of musculoskeletal models to scaled-generic knee kinematic errors</td>
</tr>
<tr>
<td></td>
<td>Wen</td>
<td>Wu</td>
<td>The influence of an assistive robotic exoskeleton on upper-limb muscle and joint function</td>
</tr>
<tr>
<td>15:30-15:50</td>
<td>Tao</td>
<td>Wang</td>
<td>Manufacture of human engineered tendon in an ex-vivo bioreactor system</td>
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<td></td>
<td>Xin</td>
<td>Wang</td>
<td>The interactions between inflammatory cytokines and fibrin clot properties</td>
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<td></td>
<td>Yinghong</td>
<td>Zhou</td>
<td>The immunoregulatory properties of bone marrow mesenchymal stromal cells during osteogenic differentiation</td>
</tr>
<tr>
<td></td>
<td>Rong</td>
<td>Huang</td>
<td>New method for studying the phenotypic shift of macrophages during bone remodeling</td>
</tr>
<tr>
<td></td>
<td>Taryn</td>
<td>Saggese</td>
<td>Co-culture system to study the interactions between notochordal and mature nucleus pulposus cells exposed to hydrostatic pressure</td>
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<tr>
<td></td>
<td>Ying</td>
<td>Zhao</td>
<td>Preparation of Magnesium-Containing Biocompatible MAO Coatings on Ti6Al4V Alloy</td>
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<tr>
<td></td>
<td>Matthew</td>
<td>Oldakowski</td>
<td>Feasible expansion size of a novel expandable fastener in the cervical spine</td>
</tr>
<tr>
<td>16:20-17:40</td>
<td>Xia</td>
<td>Guo</td>
<td>Inter-Transverse Process Spinal Fusion Induced by Electrical Stimulation to Dorsal Root Ganglion</td>
</tr>
<tr>
<td></td>
<td>John</td>
<td>Arnold</td>
<td>Biomechanical effects of lateral wedge insoles in people with medial knee osteoarthritis: a meta-analysis</td>
</tr>
<tr>
<td></td>
<td>Stuart</td>
<td>Callary</td>
<td>Low Wear of Highly Cross-Linked Polyethylene in Total Hip Replacement at Five Years</td>
</tr>
<tr>
<td></td>
<td>Bogdan</td>
<td>Solomon</td>
<td>Reduced Subsidence with Improved Femoral Impaction Grafting Techniques</td>
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<tr>
<td></td>
<td>Tesfaye</td>
<td>Leta</td>
<td>The outcome of unicompartamental knee arthroplasties after aseptic revision into total knee arthroplasties a comparative study of 768 total knees and 578 uni knees revised to total knees reported to the Norwegian Arthroplasty Register (1994-2011).</td>
</tr>
<tr>
<td></td>
<td>Claire</td>
<td>Jones</td>
<td>Comparison of patient outcomes for classic and angiosome-sparing approaches to reduce and stabilise tibial plateau fractures</td>
</tr>
<tr>
<td></td>
<td>Kishore</td>
<td>Rajendran</td>
<td>Metal artefact reduction in orthopaedic implants using mars spectral CT</td>
</tr>
</tbody>
</table>

**Afternoon coffee & tea and poster viewing**

**18:00-**

**Young investigators BBQ**

*Cafe 85, Auckland Medical School*
Day 2 (Saturday October 3) - Faculty of Medical and Health Sciences, University of Auckland, 85 Park Road, Auckland

<table>
<thead>
<tr>
<th>08:00-08:15</th>
<th>Coffee &amp; tea</th>
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<tbody>
<tr>
<td>08:15-09:05</td>
<td><strong>ECR award final</strong></td>
</tr>
<tr>
<td>Session chair: Prof Taylor</td>
<td>Geoffreys Handfield</td>
</tr>
<tr>
<td>Ju Zhang</td>
<td>Rapid Lower Limb Geometry and Muscle Insertion Estimation from Motion-Capture Landmark</td>
</tr>
<tr>
<td>Melissa Ryan</td>
<td>Yield torque is coincident with clinical tightening torque</td>
</tr>
<tr>
<td>Benjamin Schon</td>
<td>Quantification of articular cartilage health using MARS spectral computed tomography</td>
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<thead>
<tr>
<th>09:05-09:35</th>
<th><strong>Keynote: Prof Hala Zreiqat</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Session chair: Prof Taylor</td>
<td>“Development of unique ceramic scaffolds and nanoparticles with versatile modular platform for growth factor and drug delivery into bony environments”</td>
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<table>
<thead>
<tr>
<th>10:00-11:00</th>
<th><strong>PhD award final</strong></th>
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</thead>
<tbody>
<tr>
<td>Session chair: A/Prof Besier</td>
<td>Audrey Chan</td>
</tr>
<tr>
<td>Bryant Roberts</td>
<td>Regional variations in proximal tibia bone microarchitecture and joint loads in endstage knee osteoarthritis</td>
</tr>
<tr>
<td>Naveen Mekhileri</td>
<td>A biofabrication system for 3D assembly of micro-tissues for cartilage regeneration</td>
</tr>
<tr>
<td>Pavan Teja Devanaboyina</td>
<td>Does tendinopathy affect modular control of locomotion in rabbits</td>
</tr>
<tr>
<td>Young Jung No</td>
<td>Physico-chemical characterization of an injectable and resorbable strontium and zinc-doped calcium silicate phosphate cement</td>
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<thead>
<tr>
<th>11:00-12:15</th>
<th><strong>ANZORS AGM</strong></th>
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<tbody>
<tr>
<td>All delegates welcome (and encouraged) to attend</td>
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**Day 3 (Sunday October 4) - Faculty of Medical and Health Sciences, University of Auckland, 85 Park Road, Auckland**

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<th>Time</th>
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<td>Saulo Martelli</td>
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<td>A new finite-element software pipeline for the micro-structural analysis of the proximal femur</td>
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<td>Claire Jones</td>
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<td>Biomechanical evaluation of a self-supported one-handed lifting technique in healthy subjects</td>
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<td>Mousa Kazemi</td>
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<td>A computational framework to predict tibiofemoral joint kinematics</td>
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<td>Mathew Rawlings</td>
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<td>A Biomechanical Evaluation of a Novel Hybrid Approach to Midshaft Clavicle Fixation</td>
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<td>Podium 5</td>
<td>Kent Algate</td>
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<td>Polyethylene Particle-induced Osteoclast Formation Suppressed by Histone Deacetylase Inhibition</td>
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<td>Emily Hargrave-Thomas</td>
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<td>Early Osteoarthritis: mechanical and structural changes to the underlying bone and calcified cartilage</td>
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<td>Ding Yue</td>
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<td>The mechanism of SHP in macrophage induced aseptic joint loosening by wear particles in vitro</td>
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<td>Aswin Beck</td>
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<td>Proteomic investigation of early osteoarthritic tissue degeneration</td>
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<td>Oral posters 3</td>
<td>Christopher Miller</td>
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<td>A multiscale modelling approach to predict cartilage growth in scaffolds: a computational and experimental approach</td>
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<td>Marco Schneider</td>
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<td>Rosidah Ab Lazid</td>
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<td>Comparison between the cancellous screws pullout strength at three levels of tightening torque in the human femoral head</td>
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<td>Claire Jones</td>
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<td>Towards a model of the paediatric spine: characterising skeletal maturity, size and mechanical properties of immature sheep vertebrae</td>
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<td>Melissa Ryan</td>
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<td>How tight is tight enough?</td>
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<td>Dhara Amin</td>
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<td>The effect of six degree of freedom loading on the in-vitro compressive recovery properties of human lumbar spine segments</td>
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<td>Anthony FitzPatrick</td>
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<td>Total hip arthroplasty implant condition assessment through acoustic emission monitoring</td>
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<td>William Robertson</td>
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<td>Body segment parameter estimation using Hatze’s geometric model</td>
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<td>Giuliano Lambert</td>
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<td>Musculoskeletal model sensitivity to stereophotogrammetry skin artefacts</td>
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<td>Wilson Fok</td>
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<td>Samantha Rodrigues</td>
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<td>A structural investigation of the annulus-endplate junction in the ovine intervertebral disc</td>
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<td>Silvia Trichilo</td>
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<td>A computational model of the dual action of PTH in postmenopausal osteoporosis based on intercellular regulation of osteoblast apoptosis</td>
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<td>Fatemeh Malekipour</td>
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<td>Mechanical properties of third metacarpal subchondral bone in thoroughbred racehorses</td>
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<td>Xiaoli Zhao</td>
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<td>Strontium promotes the asymmetric division of MSC during the osteogenic differentiation</td>
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<td>ANZORS-China 2</td>
<td>William</td>
<td>Lu</td>
<td>The injectable biomaterials and their future development</td>
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<td>Session chair: W/Prof Xu</td>
<td>Jillian</td>
<td>Cornish</td>
<td>Lactoferrin promotes bone healing in a rat critical-sized calvarial defect model</td>
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<td>Lunch &amp; poster viewing (poster viewing will take place between 12:55-13:35)</td>
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<td>Oral posters 4</td>
<td>Mieke</td>
<td>Nickien</td>
<td>The mechanical significance of fibrillar-level de-structuring in early cartilage</td>
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<td>Session chair: Dr Ackland</td>
<td>Joshua</td>
<td>Workman</td>
<td>The effect of degeneration on articular cartilage vulnerability during impact</td>
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<td>Andi</td>
<td>Liu</td>
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<td>Assessment of changes in spatial heterogeneity of bone tissue mineral density</td>
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<td>Shasha</td>
<td>Yeung</td>
<td>Age-related shape characteristics in the equine fetlock joint</td>
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<td>Alessandro</td>
<td>Timmi</td>
<td>On the use of coloured markers to enhance KINECT V2 performance for feet tracking</td>
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<td>15:15-16:30</td>
<td>Afternoon coffee &amp; tea and poster viewing</td>
<td>Kelvin</td>
<td>Yeung</td>
<td>3D Porous Polycaprolactone-based Magnesium Scaffold for Bone Tissue Engineering</td>
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<td>Session chair: Prof Lu</td>
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<td>In Vitro Immunogenicity Testing for Novel Bone Graft Scaffolds</td>
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<td>Ryan</td>
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<td>In vitro and in vivo evaluation of phb-hv as a potential scaffold for bone</td>
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<td>Zufu</td>
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<td>Utilizing exosomes from adipose tissue-derived mesenchymal stem cells for bone</td>
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<td>Bryant</td>
<td>Roberts</td>
<td>Systematic mapping of proximal tibia subchondral bone microarchitecture in endstage</td>
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<td>16:30-16:45</td>
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Abstracts
DAY 1

KEYNOTE 1 – Dr Lin Han
UNCOVERING MURINE KNEE JOINT FUNCTION AND PATHOGENESIS VIA NANOMECHANICS

1Basak Doyran, 2Wei Tong, 3Qing Li, 2Haoruo Jia, 3Ling Qin and 4Lin Han

1School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, United States
2Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, United States
Email: lh535@drexel.edu

INTRODUCTION
Murine models are widely used for investigating the pathogenesis and progression of knee osteoarthritis (OA), owing to its low cost, short life-span and availability of genetic modification [1]. Current evaluation of murine OA relies on semi-quantitative signs measured by histological [2] or radiological [3] assays. These assays can detect tissue-level symptoms of joint abnormality. However, they do not allow us to identify alterations in prior to macroscale structural changes, or, to measure mechanical properties that are directly relevant to joint biomechanical functions. In this study, we demonstrate the potential of using atomic force microscopy (AFM)-based nanoindentation to evaluate the nanomechanical characteristics of murine cartilage after surgery-induced post-traumatic OA.

METHODS
To introduce post-traumatic OA, destabilization of the medial meniscus (DMM) surgery was performed on the right hind knee of 12-week old mice. The Sham control surgery was performed on the left hind knee of the same mice, following the procedures described previously [4]. Mice were then euthanized at 3 days to 12 weeks after surgeries. Both lateral and medial condyles were harvested for AFM-nanoindentation test performed on cartilage surfaces in PBS. Hertz model was applied to the loading F-D curves to calculate the effective indentation modulus, $E_{\text{ind}}$. In addition, whole joint histology was performed at 4-12 weeks after the surgery. Based on histology, the modified Mankins scores were calculated, as described previously [5].

RESULTS AND DISCUSSION
This study demonstrates nanoindentation is a more sensitive, function-relevant tool to study OA in mice (Fig. 1). For medial cartilage after surgeries, decrease in $E_{\text{ind}}$ appears as early as 1 week after DMM, preceding the macroscopic signs measured by histology (8-12 weeks after). In addition, lateral side cartilage also shows significant decrease in modulus at 4 weeks after, despite that histology did not detect any signs. This much earlier appearance in mechanical symptoms can be due to the fact that mechanical properties are highly sensitive to both composition and structure of ECM. The modulus of cartilage superficial layer is governed by both the concentration and hierarchical structure of its major matrix constituents, i.e., transversely aligned type II/IX/XI collagen fibrillar mesh, and proteoglycans including aggrecan and lubricin [6]. It is likely that the mechanical instability introduced by DMM disrupts superficial layer nanostructure before leading to inflammatory chondrocyte responses and later on macroscopic OA symptoms. As a result, the mechanical weakening could be a direct manifestation of the aberrant nanostructure resulted from early OA molecular events. In contrast, at late stage of 12 weeks post-surgery, a significant increase in $E_{\text{ind}}$ was observed on the medial side. This observation can be explained by the substantial loss of cartilage detected by histology, which exposes the deeper cartilage layer, which has higher proteoglycan concentration and is stiffer than the superficial layer. Thus, the late stage results suggest it is necessary to combine nanomechanical tools with conventional techniques for a systemic investigation of OA progression.

CONCLUSIONS
This study demonstrated the potential of using nanomechanical tools to understand the mechanical function of murine tissues, and to detect early signs of osteoarthritic degradation that may precede macroscopic signs.

ACKNOWLEDGEMENTS
This work is supported by the National Institutes of Health (AR066824 to LH, and AR060991 to LQ).

REFERENCES
PODIUM 1
Identifying a role for the BH4 domain of Bcl2 in bone homeostasis
Jennifer Tickner¹, Benjamin Mullin², Dian Teguh¹, Gaurav Jadhav¹, Jiaker Xu¹

¹School of Pathology and Laboratory Medicine, The University of Western Australia, WA
² School of Medicine and Pharmacology, The University of Western Australia, WA
email: jennifer.tickner@uwa.edu.au

INTRODUCTION
Pathological bone loss induced by excessive osteoclast formation, and/or a failure in osteoblastic bone formation, is the major mechanism underlying many debilitating bone diseases, including osteoporosis. A better understanding of the root cause of bone loss, and the molecules involved, is vital for an improved therapeutic strategy against bone diseases. To gain insights into the molecular genetics and mechanisms of bone loss, we have screened for bone phenotypes in chemical (ENU)-induced mutant mice to identify key molecules that regulate bone homeostasis. We have uncovered a mouse line which carries a S24P mutation in the BH4 domain of the Bc12 gene, and exhibits bone loss. Bcl2 was initially identified as an inhibitor of apoptosis; however, new roles including inhibition of autophagy, regulation of calcium homeostasis at the endoplasmic reticulum, and cell cycle regulation through p53 have recently been described. Global knockout of Bcl2 in mice results in reductions in bone formation; however, the specific role of Bcl2 in bone is yet to be completely understood. This project aimed to characterise the phenotype of the mutant mice and correlate variation in the BCL2 gene in humans with bone mineral density (BMD) in human populations.

METHODS
Bcl2 BH4 mutant mice were generated by the Australian Phenomics Facility at the Australian National University. Bone structure was analysed using microcomputed tomography with a voxel resolution of 9μm. Bone histomorphometry was performed using BIOQUANT software. In vitro osteoclast and osteoblast cultures were used to assess cell differentiation and function. Analysis of common variation in the human BCL2 gene (+/- 10 Kb) was performed using the publicly available GEFOs (GEnetic Factors for Osteoporosis) consortium dataset.

RESULTS AND DISCUSSION
MicroCT analysis of 3 month old Bcl2 BH4 mutant mice showed changes in trabecular bone parameters, with significant reductions in bone volume/tissue volume and trabecular number relative to wild type littermates. Changes in cortical bone parameters were also observed, with the cortical bone perimeter being reduced in both male and female mutant mice. In 6 month old mutant mice the trabecular bone changes were more severe in both male and female mutants. Bone histomorphometry confirmed the microCT observations and revealed a small but significant increase in osteoclast number per bone surface in mutant mice. In vitro cell culture revealed reduced osteoblastic bone formation in vitro from mutant bone marrow stromal cells. Interestingly, the bone forming activity of mature osteoblasts derived from calvaria and long bones was not affected; however, the ability of mutant osteoblasts to support osteoclast formation was reduced in co-culture experiments. The numbers of osteoclasts generated from mutant BMM in vitro varied depending on cell culture conditions. Mutant bone marrow cells responded poorly to stress, with reduced survival following reanimation. Consistent with alterations in survival under stress, levels of Beclin 1 were reduced in the mutant osteoclasts, suggesting changes to autophagy pathways. Furthermore, changes in cellular senescence markers in bone marrow cells were observed suggesting that the BH4 domain of Bcl2 is critical for cell fate decisions in bone cells governing autophagy and senescence. These processes are critical for the maintenance of bone homeostasis, particularly during ageing.

CONCLUSIONS
We have identified mutations in the BCL2 gene that have direct effects on bone cell function and BMD in both mice and humans. Our analysis of the human BCL2 gene suggests that enhanced BCL2 gene expression is associated with reduced BMD. This suggests that our Bc12 BH4 mutation may result in a gain of function, at least in bone cells. Further study is required to understand how changes in Bcl2 activity impact specific cellular functions in bone cells.

ACKNOWLEDGEMENTS
This project was supported by a University of Western Australia Research Development Award. Jennifer Tickner’s and Benjamin Mullin’s salary were supported by Raine Research Priming Grants.
Protein kinase C delta (PKC δ) null mice exhibit structural alterations in articular surface, intra-articular and subchondral compartments

Jiake Xu¹, Jian-Ping Wu², Bo He¹, Dian Teguh¹, Thomas Brett Kirk², Jennifer Tickner¹

¹School of Pathology and Laboratory Medicine, The University of Western Australia, WA
²Department of Mechanical Engineering, Curtin University, WA
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INTRODUCTION
Structural alterations in intra-articular and subchondral compartments are hallmarks in osteoarthritis (OA), a degenerative disease that causes pain and disability in the aging population. Protein kinase C delta (PKC δ) plays versatile functions in cell growth and differentiation, but its role in the articular cartilage and subchondral bone is not known. In this study, we utilised PKC δ KO mice to examine the role of PKC δ in the structural integrity of articular cartilage, and the bone/cartilage interface.

METHODS
Histological analysis including alcian blue, safranin O staining and Fluorochrome labelling were used to reveal structural alterations at the articular cartilage surface and bone-cartilage interface in PKC δ KO mice relative to WT littermates. The morphology and organization of chondrocytes were studied using confocal microscopy. Glycosaminoglycan content was studied using micromass cultures of chondrocytes derived from the cartilage of PKC δ KO and WT mice.

RESULTS AND DISCUSSION
We uncovered atypical structural demarcation between articular cartilage and subchondral bone of the PKC δ KO mice. Histological analyses revealed a thickening of the articular cartilage and calcified bone-cartilage interface, decreased safranin O staining accompanied with an increase in the number of hypertrophic chondrocytes in the articular cartilage in PKC δ KO mice. Interestingly, loss of demarcation between articular cartilage and bone was concomitant with irregular chondrocyte morphology and arrangement (Figure 1). Consistently, in vivo calcein labeling assay showed an increased intensity of calcein labeling in the interface of the growth plate and metaphysis in PKC δ KO mice. Furthermore, in vitro culture of chondrocyte micromass showed a decreased alcian blue staining of chondrocyte micromass cultures from the PKC δ KO mice, indicative of a reduction in the level of glycosaminoglycan production.

CONCLUSIONS
Our data imply a role for PKC δ in the osteochondral plasticity of the interface between articular cartilage and the osteochondral junction. Understanding the role of PKC δ in the pathological changes at the osteochondral junction may help to explore potential therapeutic applications for OA.

ACKNOWLEDGEMENTS
This study was supported by the NHMRC. We are grateful to Professors Keiichi I. Nakayama, and Keiko Nakayama for providing us with the PKC δ KO mice. The authors would also like to acknowledge the support of an NHMRC fellowship to Dr Jian - Ping Wu (ID 404179).
Differential response of notochordal and mature nucleus pulposus cells to pathological stimuli

Taryn Saggese, Kelly R. Wade, Ashvin Thambyah and Sue R. McGlashan

1Department of Anatomy with Radiology, University of Auckland, New Zealand
2Department of Chemical and Materials Engineering, University of Auckland, New Zealand

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INTRODUCTION

The nucleus pulposus of the intervertebral disc (IVD) contains two distinct cell types, notochordal cells (NC) and mature nucleus pulposus cells (MNP)1. The ratio of these cell types varies with species, age and health, with higher proportions of NC cells being associated with proteoglycan-rich healthy NP tissue, while MNP cells are associated with more fibrous tissue, which is prone to structural failure1. Large animals such as cattle and humans retain < 25% NC cells into adulthood2. In vivo, nucleus pulposus cells are exposed to dynamic hydrostatic pressure. Mechanical stress from high pressure loading is believed to be an initiating factor in disc degeneration3,4. High loading in the spine also increases the thickness of the cartilaginous endplates of the IVD which results in decreased nutrient transport into the disc5. To determine how each cell type responds to these key pathological stimuli, this study used NC and MNP cells isolated from same bovine caudal disc and exposed them to low and high magnitude loading, glucose restriction, and the combination of both.

METHODS

Sequential mechanical and enzymatic digestion was used to isolate NC and MNP cells from the same IVD. Isolated cells were seeded in alginate beads to maintain 3D microarchitecture. Cells were cultured in media-filled soft plastic pouches, to ensure equal distribution of pressure. Using a custom-built hydrostatic pressure vessel, cells were exposed to 24 h of oscillating low (0.4-0.8 MPA, 1Hz) or high (1.6-2.4 MPA, 1Hz) pressure, in media containing physiologically normal (5.5 mM) glucose. Additional experiments exposed both cell populations to low glucose (0.55 mM) media under high pressure loading. Samples were cultured under atmospheric pressure as controls. Cells were assayed for viability, GAG production, ECM gene expression and NC cell phenotypic marker expression.

RESULTS AND DISCUSSION

Across all conditions, NC cells expressed significantly greater amounts of proteoglycan than MNP cells. Low pressure loading resulted in an increase in GAG production for MNP cells while high pressure loading resulted in a significant decrease in aggrecan gene expression. Glucose restriction alone did not affect MNP ECM gene expression; however the combination of glucose restriction and high pressure resulted in decreased aggrecan and collagen II expression (Fig. 1).

NC cells did not significantly alter their ECM expression in response to either stimulus (Fig. 1).

CONCLUSIONS

MNP cell ECM expression was adversely affected by high pressure loading and this effect was increased under glucose restricted conditions. NC cells were unaffected by pressure. These results provide a possible explanation as to why animals that retain NC cells do not develop disc degeneration.

ACKNOWLEDGEMENTS

This work was funded by the Auckland Medical Research Foundation (AMRF) and School of Medical Sciences, University of Auckland.

REFERENCES

DIET-INDUCED OBESITY, EXERCISE, ADVANCED GLYCATION END-PRODUCTS: A LOVE-HATE TRIANGLE IN OSTEOARTHRITIS

1Lennex H Yu, 2Elwyn C Firth, 1,2Samuel S Haysom, 3Sophia Leung, 2Mark H Vickers, 1Sue R McGlashan.

1Department of Anatomy with Radiology, School of Medical Sciences, The University of Auckland, NZ
2The Liggins Institute, School of Medical Sciences, The University of Auckland, NZ
3Auckland Bioengineering Institute, The University of Auckland, NZ
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INTRODUCTION
Advanced glycation end-products (AGEs) are a heterogeneous group of carbohydrate-binding proteins, which causes degeneration through intra- and extracellular mechanisms. AGEs accumulate in tissues and crosslink with proteins. AGEs increase human cartilage stiffness and reduce its ability to resist compressive loading [1]. AGEs also interact with cellular receptors and induce upregulation of proinflammatory cytokines [2]. Furthermore, AGE formation occurs both exogenously (e.g., heated food) and endogenously (e.g., lipid peroxidation in vivo) [3]. Studies have shown that serum and tissue AGEs are increased by high fat diets and reduced by exercise in humans and rodents [4,5,6]. However, it is not known how high fat diets and/or exercise affect articular cartilage in terms of AGE accumulation and cytokine expression. This study aims to test the hypothesis that high fat diet results in increased systemic AGE accumulation and catabolic cytokine expression, which may be ameliorated by exercise.

METHODS
Upon weaning, 40 male Sprague-Dawley rats were randomized to 4 groups according to diet and physical activity: 1. control chow diet, sedentary; 2. High-fat diet, sedentary; 3. control chow diet, exercised (free access to a running wheel); and 4. High-fat diet, exercised until the age of 120 days. Following euthanasia, liver and kidney were sampled to measure AGE content using an auto-fluorescence assay [8]. To assess the effect of AGEs on cartilage inflammation, freshly isolated humeral heads were cultured as explants at 37°C and 5% CO2 for 48 hours and either exposed to 100µg/ml AGE-BSA or BSA. Media was collected at 24 and 48 hours, and assessed for IFN-γ, IL-1α, IL-4, IL-10 and TNF-α using the BD cytokine bead array. Mixed model and ANOVA was used to compare the baseline cytokine levels and the change over time between groups, respectively.

RESULTS AND DISCUSSION
AGE auto-fluorescence was greatest in high fat-exercised rats group in the kidney (Figure 1A). Cartilage explant culture showed no significant change in proinflammatory cytokines at baseline or after exposure to AGE-BSA. However, anti-inflammatory IL-10 was greatest at baseline in the high-fat-exercise group (P=0.01; Figure 1B), while a positive response against AGE was observed in control-exercised group (P=0.052; Figure 1C). These data suggest that exercise and high fat diet induce IL-10 secretion in cartilage, and the exercise rats on control diet have the ability to overcome proinflammatory AGEs.

CONCLUSIONS
Diet and exercise are associated with tissue AGE accumulation. While no proinflammatory cytokine response due to high fat diet was found, anti-inflammatory response was induced by exercise.

ACKNOWLEDGEMENTS
This project is funded by Arthritis New Zealand and the School of Medical Sciences, The University of Auckland.

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Figure 1: (A) AGE auto-fluorescence in kidney. (B) Explant culture baseline IL-10. (C) Explant culture IL-10 change after 24h AGE exposure. *p<0.05.
INTRODUCTION

Obesity is strongly correlated with the development of osteoarthritis (OA). Obese adolescents show signs of knee pain and disinclination to exercise, because of progressively increasing musculoskeletal discomfort, injuries, inefficient body mechanics and reduction in mobility\(^1\),\(^2\). Alarmingly, some very obese children and adolescents have knee cartilage lesions\(^3\). It was recently suggested that OA in obese people develops independently of the mechanical burden of excess weight-bearing on joints. This study aimed to test the hypothesis that rats fed a high-fat diet from weaning until 4 months of age will have an elevated systemic pro-inflammatory state, associated with significantly abnormal cartilage morphology, cartilage matrix and tissue enzyme expression and that exercise can ameliorate such inflammation and its downstream effects on joint health.

METHODS

Upon weaning, 40 male Sprague-Dawley rats were randomized to 4 groups according to diet and physical activity: 1. control chow diet, sedentary; 2. High-fat diet, sedentary; 3. control chow diet, exercised (free access to a running wheel); and 4. High-fat diet, exercised until the age of 120 days. Body composition and % body fat were assessed using dual-energy X-ray absorptiometry analysis. Serum plasma cytokines (IL-1\(\alpha\), IL-4, IL-10, IFN-\(\gamma\), TNF-\(\alpha\)) were assessed using the BD cytokine bead assay. Freshly isolated patello-femoral joints were fixed, decalcified, embedded in paraffin wax, and sagitally sectioned. Sections were scored (OARSI grading\(^5\)) for joint cartilage defects. Chondrocyte viability was assessed in freshly isolated left patellae stained with calcine AM/ethidium homoemider and imaged using fluorescence confocal microscopy, and % viability was calculated using Metaxpress image analysis software.

RESULTS AND DISCUSSION

There was a significant difference in % body fat between groups (p<0.001), with greatest % body fat in rats on high-fat diet (Fig. 1A); control exercised animals were the leanest (less than 12% body fat). In plasma, IFN-\(\gamma\) concentration in sedentary high fat-fed rats was significantly greater compared to exercised rats on a control diet (p=0.01) (Fig. 1B); plasma TNF-\(\alpha\) concentration was greater in high fat-sedentary animals than in control-exercised rats (p=0.01) (Fig. 1C). Preliminary analysis reveals higher chondrocyte death in both exercise groups compared to non-exercise groups, irrespective of diet.

CONCLUSIONS

High fat diets increase % body fat which is reduced with an exercise intervention. The high fat diet induced an increase in serum plasma concentrations of TNF-\(\alpha\) and IFN-\(\gamma\), which was moderately reduced by exercise. These preliminary results suggest that exercise could be a method of reducing the systemic inflammatory effects of a high-fat diet in young individuals.

ACKNOWLEDGEMENTS

This project was funded by Arthritis New Zealand and the School of Medical Sciences, University of Auckland. S.H is a recipient of a Gravida postgraduate Masters scholarship.

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ANZORS – CHINA 1
It is known that magnesium (Mg) is the eighth most common element in the crust of the earth and now attracts great attention to become biodegradable or biocorrosive medical implants for both cardiovascular and orthopaedic applications.

In orthopaedics, Mg and its alloys are mainly considered suitable for degradable bone implants with good initial stability that avoid a second surgical procedure to remove the temporary metallic parts for fixation after the tissue has sufficiently healed, apart from lowering overall associated health care costs. Safety concerns are also raised although Mg dissolution is unlikely to have adverse or side effects since Mg is the fourth most plentiful cation in the human body, including involvement in the formation of biological crystal apatite; it is also a co-factor for many enzymes and stabilizes the structures of DNA and RNA; beneficial from a physiological standpoint, since Mg deficiencies in human body will result in disorders of metabolic organs and cardiovascular system as well.

The author’s group is conducting R&D of orthopaedic implants, in collaboration with biomaterial scientists for developing Mg and its alloys as biocorrosive orthopaedic implants and investigating their bone stimulation effects physiologically and biologically using both in vitro and in vivo preclinical experimental models. Human pilot or Phase I studies are also conducted to investigate its biosafety as well as its efficacy for adequate orthopaedic indications.

The presentation generated from this R&D program on Mg or its alloys is based on a translational R&D model and roadmap, i.e. from Observations to Mechanisms and then to Proof-of-Concept before clinical tests, followed by clinical trials. Testing standards and guidelines are also essential for this translational roadmap and the author’s group has also been working on necessary modification for current ISO standard for testing cytotoxicity relevant for biodegradable implants.

Acknowledgement: This research program is funded by Hong Kong RGC Collaborative Research Fund (CRF 2014/2015, C4028-14GF), SMART Program of Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, the Chinese University of Hong Kong supported by Lui Che Woo Foundation Limited, and CAS-Croucher Founding Scheme for Joint Laboratories (Ref. CAS 14303).
INTRODUCTION
Re-tear after surgical repair of rotator cuff tendon is common (up to 45%). Augmentation of repair with a biological scaffold may improve long-term outcomes, but commercial products often induce inflammation due to remnant DNA and lipids. This study aimed to produce a biocompatible scaffold suitable for use in tendon repair.

METHODS
Australian sourced animal materials were investigated for physical, biochemical and biological properties relevant for use in tendon augmentation. A patented technology for the preservation of mechanical properties of type I collagen bundles was developed and the Collagen CelGro™ SmartGraft® for tendon augmentation was manufactured according to GMP standards. Standard tests for a Class III Medical Device were used to evaluate the suitability of the biologic scaffold for surgical applications in humans. Cadaver studies were carried out to develop the surgical method for repair of rotator cuff tendon tears before development of clinical trials.

RESULTS AND DISCUSSION
CelGro™ SmartGraft® contains pure porcine derived collagen with well-preserved type I collagen bundles. Preclinical examination showed that CelGro™ SmartGraft® integrates well into host tissue and is well tolerated with mild, transient foreign-body response resolving with biodegradation and absorption. No evidence of systemic immune reaction or implant site infection was observed. The native porous structure formed by the random deposition of bundles, similar to electrostatically spun fibres forms an essential environment for cell attachment, proliferation and migration.

To test the suitability of the CelGro™ SmartGraft® for rotator cuff tendon repair, cadaveric studies were conducted. A minor-open surgical procedure was used. The graft was inserted down the lateral cannula while pulling the free ends of the STIK suture stored inside the posterior and anterior cannulas. The free ends of the anchored STIKS were tensioned to remove slack, causing the graft to unfold in the joint. At the completion of the repair, the graft was firmly attached to bone laterally and to the remnants of the native tissue medially, posteriorly, and anteriorly. Fibrin glue was injected into the graft to create a biological chamber for the induction of tissue repair.

CONCLUSIONS
We have developed a collagen biologic scaffold for augmentation of rotator cuff tendon tear repair. Pre-clinical studies of the scaffold show that it displays sound mechanical properties, remains flexible and is biocompatible. Clinical trials are planned to obtain further safety, efficacy and feasibility data.
ORAL POSTERS 1
SURGERY, COST-EFFECTIVENESS, AND MEDICARE
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INTRODUCTION
Australia pioneered the use of economic evaluations in making health policy decisions. In 2015, this is particularly pertinent as an increasingly constrained Federal health budget and global increases in surgical expenditure have led to uncertainty about the marginal benefit of new surgical technologies. At the same time, a perception persists that financial impacts are a significant determinant of public funding, prompting questions of political pressure in decision-making. In Australia, the Medical Services Advisory Committee (MSAC) is responsible for considering the cost-effectiveness of surgical technologies for funding on the Medical Benefits Scheme (Medicare). As such, we aimed to assess the influence of economic evaluations on MSAC recommendations for Medicare funding.

METHODS
We performed a cohort study of surgical technology assessments prepared for MSAC from 1998-2013. For each assessment, qualitative conclusions regarding MSAC’s assessment of the evidence for safety, effectiveness and cost-effectiveness were classified as being favourable, equivocal or unfavourable. We then documented the corresponding recommendation by MSAC to support or not support Medicare funding. The chi-square test was used to analyse the relationship between MSAC’s recommendation to support or not support MBS funding, and the qualitative conclusion. The level of significance was set at p<0.05.

RESULTS AND DISCUSSION
We identified the assessment of 46 surgical technologies across 60 indications. A significant relationship was found between MSAC’s assessment of the cost-effectiveness of a surgical technology and MSAC’s recommendation to the Minister to support or not support Medicare funding. The chi-square test was used to analyse the relationship between MSAC’s recommendation to support or not support Medicare funding, and the qualitative conclusion. The level of significance was set at p<0.05.

The relationship found between MSAC’s assessment of the cost-effectiveness of a surgical technology and MSAC’s recommendation to support or not support MBS funding, suggests that, prima facie, MSAC exercises evidence based judgments in making its recommendations. However, the association amongst recommendations against MBS funding, equivocal or unfavourable evidence for cost-effectiveness, and otherwise equivocal or unfavourable evidence for safety and/or effectiveness, raises the possibility of type II errors. This is especially noteworthy in surgery, given the historical difficulties cited in the gathering of higher evidence of safety and effectiveness.

CONCLUSIONS
To minimise stakeholder concerns over any perceived inconsistencies between evidence and MSAC’s recommendations, further investigation should be undertaken to how economic evaluations are used in maximising the net benefit of Australian healthcare expenditure.

REFERENCES
Absorbable Polydioxanone (PDS) suture provides fewer wound complications in acute Achilles tendon rupture repair.

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INTRODUCTION
We prospectively studied acute Achilles tendon rupture in patients over a 2 year period and reviewed the causes, clinical outcomes and complications.

METHODS
There were 53 consecutive patients included in our study, who attended a district general hospital with an acute Achilles tendon rupture. We prospectively collected their bio-data, medical history, cause of injury and mode of treatment. They had a minimum follow up period of 6 months, clinical outcome was measured using Boyden score and complications incurred were recorded.

RESULTS AND DISCUSSION
We randomized the 53 patients into two groups according to admitting consultant. Out of fifty three 53 Achilles tendon ruptures, 19 patients were repaired using Polyester (Ethibond) and 34 patients were repaired using Polydioxanon (PDS) suture. The functional Boyden score, in the excellent category, was greater for patients treated in the PDS suture group compared to the Ethibond suture group, this was statistically significant p<0.05.

There were 6 surgical infections of the operative site and all infected cases had a suture repair using the polyester (Ethibond) suture. There were no infections in the polydioxanon (PDS) repair group. This was statistically significant P<0.05, when the two suture groups were compared for post op infections.

CONCLUSIONS
On the basis of this study, we suggest that the surgical repair of the Achilles tendon with monofilament non-braided absorbable suture polydioxanone (PDS) is superior, in respect of lower post-operative wound complications.
A PROSPECTIVE COMPARISON OF THE ANTERIOR STABILITY AND KINEMATICS OF FOUR- AND FIVE-STRAND HAMSTRING TENDON AUTOGRRAFT RECONSTRUCTED KNEES

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INTRODUCTION
The Four-Strand (4S) Hamstring Tendon Autograft has been long established as the gold standard for surgical reconstruction of the Anterior Cruciate Ligament. Some studies have suggested larger graft diameters may provide a larger scaffold for incorporation of the graft into the bone tunnels and potentially reconstruct a greater portion of the native tibial and femoral footprints, leading to greater postoperative anterior stability of the knee and better replicating the biomechanical behaviour of the native ACL in vivo. A Five-Strand (5S) graft, using a three-strand Semitendinosus/two-strand Gracilis construct, has been proposed as a wider graft construct to enhance antero-posterior stability of the knee and produce more stable kinematics postoperatively.

AIM
To determine whether a Five-Strand Hamstring Tendon Autograft will produce better antero-posterior stability and more stable kinematics than that of the gold standard Four Strand Hamstring Tendon Autograft in ACL Reconstructive Surgery.

METHODS
30 (n=20 Four-Strand and n=10 Five-Strand) patients with planned ACL reconstructive surgery by a single surgeon were recruited for this study. The KT-1000 Arthrometer (MEDmetric, CA, USA) was used to quantify AP translation in the subjects’ knees before (T0) and after surgery at 6 (T1) and 12 (T2) weeks. The Knee KG System (EMOVI Inc, Canada) was used to quantify six-degrees-of-freedom knee kinematics during level walking at 6 and 12 weeks postoperatively. Unpaired t-tests and Mann-Whitney Non-Parametric tests were used to test for differences between graft types. Pearson’s and Spearman’s Correlation coefficients were used to correlate graft widths with measures of postoperative kinematics, anterior laxity and change in laxity over time.

RESULTS AND DISCUSSION

Anterior Stability
At 12 weeks there was significantly higher mean anterior laxity (5S=9.1±1.7mm, 4S=6.9±2.3mm, p=0.014) and mean side-to-side differences (5S=5.1±3.5mm, 4S=1.9±2.2mm, p=0.011) on Maximum Manual Test in the Five-Strand than the Four Strand group. A significantly larger positive mean change in anterior laxity (p=0.016) from 6-12 weeks was evident in the Five-Strand (1.4±0.9mm) than the Four-Strand group (-0.3±1.9mm). No significant correlations were seen between graft diameter and measures of anterior stability on KT-1000.

Kinematics
The Five-Strand group showed significantly less rotational stability (p=0.026) at Mid-Stance of the gait cycle at 6 weeks, however by 12 weeks this difference was not significant (p=0.196). At 12 weeks significant positive correlations were seen between graft diameter and better rotational stability of the knee at Heel-Strike (r=0.415, p=0.031) and Maximum Internal Rotation (r=0.456, p=0.017), regardless of graft type. Strong trends to significance were seen with correlations of graft diameter and rotational stability at Terminal Stance (r=0.379, p=0.051) and Maximum External Rotation (r=0.358, p=0.067) at 12 weeks postoperatively.

CONCLUSIONS
This study illustrated that Five-Strand Hamstring Tendon Autografts did not produce superior anterior stability of the knee when compared to the gold standard Four-Strand construct. Larger graft diameters however, were associated with better rotational stability of the knee during level barefoot walking, regardless of graft type.
INTRODUCTION
Despite high interest in osteoarthritis (OA) in sheep, accounts of natural disease are rare. Experimental sheep models have been widely used in orthopaedic research, and ovine OA may be a useful model of idiopathic human OA. This study examined the stifle (knee) joint of aged sheep.

METHODS
The 6-year-old sheep (n=40) examined were twin-born female offspring of a very large cohort [1] of Large or Small (L,S) AI-bred ewes randomly allocated to maintenance (M) or ad lib(A) pasture herbage as the sole nutritional source from pregnancy day21 (D21) to D140. The four groups of offspring had been managed together as a commercial flock at pasture from birth until 6 years of age. Immediately after slaughter the hindquarters were scanned (DXA), defleshed, and femorotibial (knee) joints stored (-20°C). Articular cartilage surfaces of proximal (Prox) and distal half of patella, axial and abaxial (Abax) surfaces of proximal tibia, femoral condyles (FC), femoral trochlea (lateral, medial ridges, trochlear groove (LTR, MT and TG) were inspected under bright light, stained with India Ink classified using a modified OARSI system, and photographed.

To determine the effect of maternal size and nutrition during pregnancy on offspring cartilage scores, a multivariate general lineal model was conducted with Size (L,S), Nutrition (M,A), and interaction (Size*Nutrition) included as fixed factors and weight as a confounder.

RESULTS AND DISCUSSION
Cartilage scores of 13 different regions are shown (Table 1). Appropriateness of GLM was tested and evidence of non-normal distribution of residuals (i.e. predicted values against observed values) was not detected in any variable. There was no significant effect of size or nutrition on any of the exposure variables (P>0.05). Effect size in some variables were medium to high (η_p^2> 0.20), indicating that lack of effect is most likely due to low group size; in all cases, post hoc statistical power was very low (<30%). We contend that the maternal environment most likely had later-life effects, and further study with greater statistical power is indicated.

There were marked differences between scores in tibial and femoral axial and abaxial sites, which coincided with non-meniscal and meniscal areas; presumably the cartilage surface is protected by the meniscus, obvious defects in which were not detected. The cartilage defects were reasonably mild, and it is unknown when such might begin. Determination of partial or full thickness cartilage loss in various lesions is ongoing. There was no evidence of these sheep being lame at or before slaughter, although others in the flock had been culled months or years earlier for various reasons, including lameness. We can draw no conclusions as to the rate of progression of AO in ageing twin-born female sheep.

CONCLUSIONS
The changes resemble those illustrated recently [2] and in contrast to experimentally induced ovine OA appear to be naturally occurring. Study of such lesions in large cohorts may be a useful addition to OA biology research.

ACKNOWLEDGEMENTS
The authors are grateful for funding awarded by the National Research Centre for Growth and Development, and for the assistance of Ms S Patel.

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Table 1: Mean ±SD group and total (T) cartilage surface scores of aged female sheep.

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DEMOGRAPHICS, MECHANISMS AND RADIOGRAPHIC ANALYSIS OF CERVICAL FACET DISLOCATION: A DECADE OF ADMISSIONS TO THE ROYAL ADELAIDE HOSPITAL

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INTRODUCTION

Traumatic cervical facet dislocation (CFD) is often associated with devastating spinal cord injury (SCI) [1]. In Australia, 50% of traumatic SCIs reported in 2008 resulted in tetraplegia, with care costs of $14.6 million p.a. [2]. Cervical facet injury is understudied in comparison to other neck trauma, and available clinical data is limited to reviews of SCI and trauma across all injury types. Improved understanding of CFD injury mechanisms is crucial to develop preventative measures and new approaches to treatment. The aim of this study was to identify associations between CFD demography, injury mechanisms, radiographic features, and neurological deficit.

METHODS

We obtained medical records and imaging (Xray, CT and MRI) for all CFD cases (including subluxation and fracture-dislocation) admitted to the Royal Adelaide Hospital (RAH) between January 2003 and December 2013. Patient demographics, and injury and management details were collected from electronic databases; however, complete data describing neurological deficit were not available, so a case-note review is in progress. A custom image analysis program was developed to quantify parameters describing extent of column disruption: vertebral translation, facet apposition, canal compromise, cord compression, and MRI signal change (indicator of SCI) [3]. Descriptive statistics (mean±SD) were obtained for the existing data and independent t-tests (α=0.05) were used for comparisons. At completion of data collection, regression models will be used to determine the parameters predictive of neurological deficit.

RESULTS AND DISCUSSION

In the study period, 232 patients (72.4% male) with CFD were admitted. Age at admission was 45.3±21.6 yr with a bimodal distribution (peaks at 20-29 and 80+ years, Fig. 1). This corresponded to a difference in primary cause of injury: motor vehicle/bike accidents (high-energy) for <60 yrs; falls (low-energy) for 60+ yrs. 55.6% of patients had completely dislocated or perched facets; half of these were bilateral. The most commonly injured spinal levels were C6/7 (37.5%) and C5/6 (25.4%). Injury to these levels resulted in the longest hospital stay (median: C6/7=13d; C5/6=12d) and most commonly resulted in spinal cord MRI signal change (C5/6=41.7%; C6/7=29.8%). The patient population for which image analysis is complete has similar demographics (N=130). CT images were available for 84/130 patients; of which 44/84 also had MRI data. Those with and without MRI signal change had an average antero-posterior (AP) vertebral translation of 34.6±26.6% and 19.1±18.9%, respectively (p=0.039). Facet apposition was lower for dislocation than subluxation (Left: 44.4±34.4% vs. 61.4±33.4%, p=0.035; Right: 39.8±35.3% vs. 64.2±26.0%, p=0.001).

CONCLUSIONS

This population of traumatic RAH CFD admissions has similar demographics and injury causation to those previously reported [1]. CFD results from high- and low-energy injury mechanisms in old and young age-groups, respectively. AP vertebral translation and facet apposition may be predictive of neurological deficit and CFD severity. This information will be used to inform the design of laboratory experiments to better understand the biomechanics underlying CFD.

REFERENCES

LOW BONE MINERAL DENSITY SUPERIOR TO LOOSE ACETABULAR COMPONENTS
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INTRODUCTION
Following total hip replacement (THR) bone surrounding the implant undergoes remodelling. Loss of the surrounding cancellous bone may lead to loosening of the prosthesis and require complex revision surgery. Quantitative computed tomography (qCT)-Assisted Osteodensitometry is a relatively new technology which can be used to investigate periprosthetic bone mineral density (BMD) following THR with high resolution, accuracy and reproducibility. The aim of this project is use qCT to investigate the regional pelvic bone mineral density (BMD) surrounding primary THRs.

METHODS
Patient Cohort
Two patient cohorts were prospectively enrolled at the Royal Adelaide Hospital and underwent primary THR for a diagnosis of osteoarthritis (OA), or revision THR for acetabular component loosening by a single surgeon (LBS).

qCT Assisted Osteodensitometry
All patients underwent pelvic qCT scan with SIEMENS Somatom Definition AS+ CT Scanner (Siemens Ltd) using a validated metal artefact reduction protocol [1-3]. Scans were performed at 3 days post-op for Primary THR patients at 1 month pre-op for Revision THR patients. A hydroxyapatite phantom (Mindways Inc., Austin, USA) was asynchronously scanned to allow calibration of patient scans. Axial reconstructions were analysed using QCT ProTM software (Mindways Inc., Austin, USA), to measure volumetric BMD mg/cm$^3$ in cancellous bone at four regions of interest, based on the models of Wright et al. and Mueller et al. [4, 5]. Identical measurements were taken in the un-operated contralateral side as a control.

RESULTS AND DISCUSSION
A similar trend in regional BMD distribution was observed in the un-operated hip of primary and revision patients, with highest BMD in the superior region in the ilium above the acetabular component. In primary patients, BMD was higher superior and posterior to the acetabular component compared to the contralateral side. BMD in these regions were also similar to those reported by Mueller et al. however BMD at the anterior region was much lower. BMD in superior and posterior to the acetabular component of revision patients was lower than that of primary patients. Kress et al. report a progressive loss of 30-40% BMD in these regions between 1-3 years but no further loss at 10 years after surgery, in cancellous BMD around stable acetabular components.

CONCLUSIONS
BMD is lower superior and posterior to a loose acetabular component, but higher in the anterior region. The trend in regional BMD distribution is similar in the un-operated contralateral hip of patients undergoing primary and revision THR. Future studies will monitor BMD changes in these patients.

ACKNOWLEDGEMENTS
Radiology Department, Royal Adelaide Hospital

REFERENCES
BIOLOGICAL AND CLINICAL EVALUATION OF INTRAOPERATIVE RETENTION OF AUTOLOGOUS CHONDROCYTES ON TYPE I/III COLLAGEN SCAFFOLD (ORTHO-ACITM) FOR CARTILAGE REPAIR

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INTRODUCTION
Matrix-induced Autologous Chondrocyte Implantation (MACI) is the second generation of ACI, wherein autologous chondrocytes are seeded onto a collagen scaffold prior to implantation. Adoption of MACI has been hampered by high production cost. Therefore, the aim of this study was to develop a more cost-effective method of this effective technique.

METHODS
Cells were seeded onto the collagen scaffold and retention measured by PKH26 staining and confocal microscopy from 7 to 120 minutes thereafter. Molecular markers of chondrocyte lineage (collagen II, aggrecan, SOX9, HAPLN1) were also compared against cells seeded for 4 days (current MACI). After chondrocyte retention and molecular profile were validated, 15 patients were recruited to receive the modified MACI treatment (Ortho-ACITM). Cartilage repair was assessed by arthroscopy or MRI and graded as excellent, good, poor or no in-fill. Repair outcome was then correlated with variables such as patient age, graft size, location and prior history.

RESULTS AND DISCUSSION
Collagen scaffolds displayed 79% cell retention at 7 minutes post-seeding, increasing to 97% cell retention after 20 minutes and 99% by 90 minutes after seeding. The molecular profile of chondrocytes seeded onto scaffolds for 20 minutes was more consistent with primary chondrocytes than those seeded for 4 days. Fifteen patients received Ortho-ACITM treatment, with the majority of patients having multiple defects (25 defects, mean 1.7 grafts per patient). Most grafts were for patella defects (36%), then medial femoral condyle and trochlea (total 80%). Good or excellent MRI outcomes were noted in 100% of grafts at a mean 25 months follow-up (n=5). Good or excellent second look arthroscopy outcomes were noted in 83% of cases at a mean 17 months follow-up (n=24). Complications directly related to the graft were noted in 29% (7/24) of cases, with all presenting as graft edge tissue overgrowth. No associations were found between repair outcome and surgical variables, although interestingly, 6 of the 7 graft overgrowth cases were graded as excellent arthroscopic repair.

CONCLUSIONS
The Ortho-ACITM technique involves seeding chondrocytes onto collagen scaffolds in theatre, a more cost-effective practice than normal MACI, wherein cells are seeded 4 days prior to implantation. Findings from in vitro studies and case series of Ortho-ACITM provide preliminary evidence that it is a safe, effective and cost-effective MACI procedure.
Identifying growth factors for improving the healing of the tendon-bone interface

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INTRODUCTION
Injuries to tendon-bone interfaces are a significant clinical problem occurring in otherwise healthy, active people. Primarily caused by overuse or trauma, these injuries significantly affect patient quality of life and are a substantial financial burden to the healthcare economy. While there are currently no clinically accepted biological treatments for improving healing of these soft-hard tissue interfaces, much research has attempted to identify growth factors capable of aiding this challenging process.

In this study, a number of factors (IGF-1, lactoferrin, PTH, TGF-β) that are known to be important in bone biology have been evaluated for their ability to be anabolic to tendon. We compare these factors to PDGF, a factor much studied in tendon repair.

METHODS
Primary tenocytes harvested from rat tails or human bicep tendons were treated with a range of factors for 48 hours. Cell growth was determined using alamarBlue® assays and collagen deposition was measured by Sirius red dye release. Gene expression analysis using real-time PCR was used to study tenocyte differentiation.

RESULTS AND DISCUSSION
PDGF was the only factor that significantly increased tenocyte cell numbers (>30%, p<0.05), while PDGF, IGF-1 and TGF-β all increased collagen production in vitro (p<0.05) (Figure 1). IGF-1 also increased the expression of tenasin-C and tenomodulin genes (both p<0.05), both important factors in the early response to tendon healing. TGF-β, meanwhile, also increased the expression of tenomodulin (p<0.05).

Both lactoferrin and PTH slightly decreased tenocyte number (~10%, p<0.05), but increased the expression of IGF-1 (lactoferrin ~2.5-fold and PTH ~10-fold).

Interestingly, while PDGF greatly increased tenocyte cell number and collagen production, it significantly decreased the expression of a number of genes important in tendon cell biology, such as biglycan, scleraxis and tenomodulin (all p<0.05).

CONCLUSIONS
Here we have shown that both IGF-1 and TGF-β have the potential to improve healing outcomes of the tendon-bone interface, due to their known roles in bone biology and their anabolic effects on tendon cells. It appears that lactoferrin and PTH hold less promise in this regard.

Interestingly, it appears that although PDGF is much studied in tendon healing, it may actually result in a de-differentiation away from the tenocytic lineage.

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INTRODUCTION
Globally, over two million bone grafting procedures are performed annually, with an estimated increase of 13% per year [1, 2]. Despite the rapid rise in demand, currently available bone grafts are becoming increasingly limited due to safety concerns, escalating costs and supply issues. Microcrystalline Hydroxyapatite Calcium (StimuCal™) is a bovine bone material with potential to be osteoconductive due to the presence of a mineral apatite phase; furthermore, StimuCal™ has a ≈22% organic component which we have previously shown to be anabolic to bone, in vitro. The aim of this study was to assess the potential of the StimuCal™ product as a whole to act as a bone graft substitute.

METHODS
A number of growth factors and the bone matrix protein, osteocalcin, were extracted from StimuCal™ and quantified using immunoassays.

The effect of StimuCal™ on human osteoblast growth was evaluated in a 3D collagen gel over a 14-day culture period using alamarBlue® assay (Invitrogen) and osteoblast differentiation assessed by analysing changes in osteoblast marker gene expression using real-time PCR.

RESULTS AND DISCUSSION
StimuCal™ contains a number of osteoinductive growth factors including IGF-1, IGF-2, total TGF-β and osteocalcin. The alamarBlue® assay demonstrated significant increases in the number of viable osteoblasts over the 14-day culture period (p<0.0001) (Figure 1).

Real-time PCR analysis demonstrated that alkaline phosphatase (Alpl) gene expression significantly increased over the 14-day culture period in both groups (p=0.0008). However, the expression of osteoblast marker genes over 14-days of culture demonstrated minimal difference between cells grown in collagen gels with or without StimuCal™.

Despite the presence of growth factors and the bone matrix protein osteocalcin, our study did not identify any additional benefit of incorporating StimuCal™ into 3D collagen gels with regards to osteoblast growth and differentiation. The lack of effect may be a reflection on the innate limitation of the in vitro assays employed in this study, which fails to replicate the complex in vivo environment required for growth factors to exert their effect. Many variables that control growth factor release and function cannot be replicated in vitro. For example, the growth factors and bone matrix proteins in StimuCal™ may only be released once the minerals are resorbed through osteoclast activity in vivo.

CONCLUSIONS
This study demonstrated that StimuCal™ is cytocompatible and has no negative effects on human osteoblast growth or differentiation.

In light of its cytocompatibility, the potential of the retained growth factors, and the low manufacturing costs required to manufacture StimuCal™, we feel that StimuCal™ still has the potential to be used as a sustainable biomaterial for bone regeneration. In vivo analysis is currently underway to explore the role of the retained growth factors in StimuCal™ as well as the safety profile of this product.

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REFERENCES
ON THE USE OF IN VIVO EQUINE DATA TO INFORM A MECHANOSTATISTICAL MODEL OF CORTICAL BONE REMODELLING

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INTRODUCTION
The equine model is highly suited to investigating human cortical bone behaviour. It has similar haversian structures; has a fetlock joint (similar to the human knee); offers the ability to measure in vivo data; and is recommended by the U.S. FDA for comparative joint research, making it a useful translational model to the human. The objectives of this study were: (1) to develop a 3D multiscale equine model of cortical bone behaviour that is informed by measured in vivo bone growth rates and bone strain; and (2) to make this model useful in practice by developing a surrogate model to link micro cortical bone behaviour to the whole bone.

METHODS
Bone biopsies taken from the dorsal cortex of the cannon bone of size 4 mm × 3.5 mm × 2 mm were imaged at 5 μm resolution using an Xradia MicroXCT machine. The cortical structures were extracted in ITK-SNAP and voxel meshed in Hypermesh for finite element simulation in Abaqus. A cortical bone remodelling algorithm was integrated [1] that used stress stimuli to initiate bone growth and bone density responses according to the 'mechanostat' developed by Frost [2]. Bone remodelling rates were adapted from biostained images taken from the equine cannon bone (Figure 1, left) during controlled periods of exercise (trot, canter, gallop, rest) with known speeds of 4 m/s, 10 m/s, 17 m/s, and 0 m/s, respectively [3]. These were linked to in vivo bone strains measured from strain gauges placed on the surface of the dorsal cortex and ranged from 2.6 μm/day (trot) to 12.7 μm/day (gallop). It was observed that horses trained at galloping speeds continued to show significant bone growth during the post-gallop rest period. This motivated the development of a 'fading memory' model, whereby recent loading histories are remembered. A 3D haversian 'cutting-cone' model was included (Figure 1, right) to describe haversian tunnelling and bone resorption. Finally, to make this model useful in practice we trained a surrogate model using partial least squares regression (PLSR) to predict micro-architecture and homogenised Young’s modulus given known whole body loads.

RESULTS AND DISCUSSION
The cortical bone model predicted the filamentary tunnelling behaviour that was primarily in the longitudinal direction of bone. Surrounding loads caused the haversian canals to oscillate in shape similar to that observed in synchrotron imaging and micro CT. The haversian canals showed merging behaviour and predicted formation of ‘super osteons’ [4] and Volkmann canal-like behaviour, suggesting that porosity increase may be due to haversian canal merging. The memory effect generated continued bone growth even after loading stimulus was removed. This was consistent with the data measured from galloping horses which continued to show significant in vivo bone growth during post-exercise rest. The surrogate model was used to predict bone shape and homogenised Young’s modulus for bone not used in the training with average errors of less than 1%.

Figure 1: (Left) biostained image depicting bone growth; (Middle) equine histological section location; (Right) microscale cortical bone remodelling.

CONCLUSIONS
The equine model (as opposed to haversian-less murine models) informed the computational model with in vivo cortical bone growth rates and bone strain. Human models only present limited end of life data with unknown loading. Our surrogate model can inform whole bone strength changes from disease, drugs and micro level effects. The results of this study are currently being developed for inclusion in the open-source IUPS Physiome project repository [5] for evaluation and sharing by the scientific community.

ACKNOWLEDGEMENTS
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REFERENCES
INTRODUCTION
The enthesis attaches ligament and tendon to bone. It is a structure of that is highly complex and involves micro- to nano-scale structural features that govern biomechanical functions at the macro-scale level. After a ligament injury, surgical reconstruction is often required to restore normal knee functions. However, despite continuous improvement in the reconstruction techniques, anatomical restoration of the enthesis is still limited. While part of the problem is a limitation in technical capability, there remains a pressing need to further understand the micro-to-nano scale level structures of the ligament enthesis.

Of interest in the current study is the multi-scale level structure of the human Anterior Cruciate Ligament (ACL) tibial enthesis. The ACL is the most frequently injured knee ligament. Various morphological studies on the ACL and its enthesis have been conducted previously, however most of them have been confined to a single scalar level. In this new study, novel experimental methods were used to investigate in detail the micro-to-nano scale structure of the human ACL tibial enthesis.

METHODS
A total of six paired ACL tibial entheses, from three cadavers, formed the basis of this study. The donors were elderly females aged at 89, 90, and 92. Ethical approval was obtained.

Specimens were chemically fixed in formalin in its fully relaxed state, mildly decalcified, and then cryo-sectioned to produce sagittal slices (~20µm thick) of the ligament-bone enthesis. The sections were then imaged in their fully hydrated state using bright field and differential interference contrast (DIC) optical microscopy. After optical microscopy examination, selective slices were treated with graded ethanol solutions, critical point dried (CPD), and sputter coated with platinum for further investigation using scanning electron microscopy (SEM).

Two decalcified specimens were also intentionally ruptured through the enthesis. To do this, an incision was made in the ligament, along the sagittal plane, before the two separated ligament bundles were pulled apart to produce a tear through the sagittal plane of the enthesis. The exposed enthesis surface was then processed for examination using SEM.

RESULTS AND DISCUSSION
A range of enthesis features were found at multiple scalar levels, and these features are summarized in Figure 1.

CONCLUSIONS
The results also suggests that the anchorage of ligament to bone is highly complex and consists of a collection of structural and compositional features at multiple scalar levels.
MEASURING THORACIC RANGE OF MOTION USING KINECT 2 AND COMPUTER VISION TECHNIQUES

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INTRODUCTION
There has been limited biomechanical examination of the static and dynamic posture of Cystic Fibrosis (CF) patients, and the range of motion (ROM) across their vertebral segments is yet to be identified. Standard clinical optical motion analysis procedures to assess postural parameters are difficult to implement and too time consuming for routine clinical use. They are particularly prohibitive for the CF population due to the general morbidity and secondary complications of the disease [1].

Microsoft Kinect for Windows V2 (Kinect 2) is a low cost marker-less motion capture device, capable of tracking 25 body joints in real-time, and has great potential for routine clinical use due to its quick and easy setup as well as its portability. However, the Kinect 2 software provides skeleton that has significant anatomical limitations in the spine, providing only three joints with restricted degrees of freedom. This limits the accuracy and usefulness of the device in estimating spinal kinematics. However, Kinect 2 provides a high definition colour stream and a high fidelity depth stream that can be processed using computer vision techniques, to obtain imaging information beyond that provided by the SDK.

OpenCV is a free open source library for real-time computer vision applications [2]. The objective of this study was to use OpenCV to apply computer vision techniques to the Kinect colour and depth streams to track a set of six passive coloured markers affixed to the spine (Figure 1a) and capture accurate spinal kinematics in real-time.

METHODS
Six coloured markers were created using 30 mm Styrofoam balls and acrylic paint. Markers were affixed to the C7, T2, T4, T6, T8, T10 spinous processes using double sided tape. The Kinect 2 colour stream was filtered using a HSV filter to isolate areas of the image matching the marker colours. The 2D location of markers was determined by detecting contours in the threshold image which satisfied certain constraints in terms of shape and area. It was then mapped to the depth stream to determine the marker location in 3D space. Marker locations during left lateral trunk bending were recorded in a trace file and Inverse kinematics (IK) was performed in Open Sim using a model of the thoracic spine (Figure 1b). ROM was determined by calculating the difference between the maximum and minimum angles of the intervertebral joints.

RESULTS AND DISCUSSION
The new algorithms developed are able to track six colour markers at 30 fps. The system was able to detect ROM of each vertebra, however ROM for some segments fell below the normal anatomical range (Figure 1c). Future iterations of the model will incorporate kinematic constraints to mimic the movement of the intervertebral joints more accurately.

CONCLUSIONS
The flexibility of the Kinect 2 data streams enables the development of ad-hoc algorithms for more advanced tracking scenarios. Future work will validate the accuracy of the tracking algorithm against gold standard, marker-based motion capture systems (e.g. Vicon) using an approach similar to that reported previously [3]. Our software will be used to track spinal ROM in a cross sectional study of CF patients of different ages and made available for download at www.kinedge.net.

REFERENCES
A COMPARISON OF STRESS IN THE LOWER LUMBAR BETWEEN THE SHEEP AND HUMAN MODEL: APPLICATION TO EVALUATION OF SPINAL FUSION IMPLANTS

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INTRODUCTION
Predictive computational models have increasingly been used alongside animal trials for evaluating the success and predicting the outcomes of orthopaedic procedures. First introduced to the field of orthopaedics in 1972, finite element (FE) models have been used for three main purposes: (i) for design and pre-clinical analysis of prostheses; (ii) to obtain fundamental knowledge regarding musculoskeletal structures; (iii) to investigate time-dependent adaptation processes such as tissue growth and bone remodeling [1]. In line with the general increase in awareness towards animal welfare, this study aims to create accurate FE model of the sheep lower lumbar vertebra to be used as a tool for orthopaedic evaluations of spinal fusion implants. By contrasting the stress patterns between human and sheep vertebra, implications of sheep orthopaedic evaluations can more accurately be translated to the human condition.

METHODS
A 3D FE model of the sheep lumbar vertebrae was developed using Computed Tomography (CT) images imaged with 0.625 mm resolution. Geometrically accurate intervertebral discs were constructed using a variant of free-form deformation technique known as “Host Mesh Fitting” [2]. The completed sheep spine model was then modified by transforming each vertebrae to form multiple postures. Using the host mesh fitting technique, the intervertebral discs were deformed accordingly. In parallel, a 3D FE model of the human spine was constructed from the Visible Human Project run by the U.S. National Library of Medicine (NLM) [3]. Weight bearing stress analyses were performed on both the human and sheep spine models using the finite element analyses software, Abaqus. Finally, to evaluate the effect of orthopaedic implants in our model, a 3D CAD model of a trabecular metal spinal fusion implant was meshed and inserted into both the human and sheep FE models.

RESULTS AND DISCUSSION
Our FE models predict higher stresses in the sheep spine as expected, with higher peaks towards the center of the vertebrae in contrast with a more dispersed stress pattern in human. The main observed difference between the patterns of stresses in the models is that the sheep spine stress pattern is more anterior and the human spine stress pattern is posterior. This is partly due to the walking pose of sheep (on four limbs) as opposed to humans (upright). Introducing the implant produced the stress-shielding patterns in accordance with Wolff’s law. Our model shows that the implant has the effect of concentrating and redirecting stress propagation towards the posterior region, an effect that would more significantly affect a human spine and should be taken into account. Furthermore, we observe lower interfacial stresses and more evenly distributed stresses for implants with larger contact areas.

CONCLUSIONS
This study demonstrates a comparative study to customize the sheep spine to human loading conditions, forming the pioneering step in using sheep spine computational models as an orthopaedic evaluation tool.

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REFERENCES
ANALYSIS OF TOTAL KNEE ARTHROPLASTY GAIT USING A MULTIPLE REGRESSION NORMALIZATION

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INTRODUCTION
Quantitative gait analysis is widely used in the evaluation of movement impairments; however, gait features characteristic of a cohort may be difficult to measure and evaluate due to between-subject differences in physical properties, as well as walking speed. To reduce this variability, normalization of gait data is frequently performed using standard dimensionless (DS) equations [1]. Statistical techniques, including linear, multiple-linear, and non-linear regression methods, have also been used for this purpose; however, none of these methods account for variations in subject physical properties and walking speed. The aim of this study was to use a multiple regression normalization (MR) method that accounts for subject age, height, body mass, gender, and walking speed, and to use this approach in gait analysis of unilateral total knee arthroplasty (TKA) patients.

METHODS
Gait data for 45 unilateral TKA patients and 31 aged-matched healthy controls were selected retrospectively from a gait database. Three dimensional gait data were acquired using an 8-camera Vicon Motion Analysis System, and two instrumented force platforms. Peak joint angles, Ground Reaction Forces (GRFs), net joint moments, and net joint powers at the hip, knee, and ankle were normalized using DS equations [1], as well as a MR approach as follows:

\[ y_i = \beta_0 + \sum_{j=1}^{p} \beta_j x_{ij} + \epsilon_i \]  

(1)

where, \( y \) represents the peak value; \( x \) represents the physical properties and walking speed; \( \beta \) represents the regression coefficients; and \( \epsilon \) represents the independent residual error. Gait features are normalized by calculating the ratio of the original and fitted values as follows:

\[ y_i^n = \frac{y_i}{\hat{y}_i} \]  

(2)

where, \( y \) is the original gait feature, \( \hat{y} \) is the best fitted value and \( y^n \) is the normalized gait feature.

Spearman’s rank order correlation coefficient (r) was used to assess the influence of subject walking speed and physical properties on gait data normalized using DS and MR. Two-tailed student’s t-tests were used to compare mean differences between the TKA and controls. Statistical significance was set at p<0.05.

RESULTS AND DISCUSSION
Normalizing gait data using DS only marginally reduced the correlations between gait data, subjects physical properties, and walking speed while normalizing using MR reduced all these correlations to weak values (\(|r|<0.3\)). Normalizing gait data using either DS or MR resulted in significantly lower first and second peaks of the vertical GRF, knee adduction moment and knee extensor powers in TKA patients compared to controls (p<0.05). However, MR identified a significantly higher peak hip extension angle (p=0.02) and peak hip flexion powers (p=0.03) in TKA patients compared to controls, which were not present after using DS (Figure 1). Greater hip extension angles and hip flexor power may be a compensatory mechanism associated with low concentric knee extensor power, or ‘quadriceps avoidance’ type gait.

CONCLUSIONS
TKA patients demonstrate greater hip extension angle and hip flexor power, and a lower knee adduction moment than healthy controls, which may be a strategy to reduce knee muscle and joint loading. However, these gait differences could not be discerned after normalization using DS due to residual correlations between subjects’ physical properties, walking speed, and the gait data. By minimizing these correlations, MR reduces the likelihood of a false negative result. The method developed may be useful in machine learning, and gait classification.

REFERENCES
Ankylosing spondylitis (AS) and rheumatoid arthritis (RA) share similar features during early disease stages of high levels of pro-inflammatory cytokine production and joint damage through osteoclast activity. However, as the diseases progress, their pathologies diverge significantly with joint destruction in RA continuing to be mediated by cytokine-driven bone joint degradation. In AS, however, the inflammation drives an osteoproliferative phenotype.

The extent of joint destruction through the cytokine/osteoclast axis in AS is significantly less than in RA with the dominant joint impact stemming from the ankylosis that can often occur as a result of the osteoproliferation. Very little is known about the progression from the initial inflammatory stages of the disease to this pathologic bone formation. There are three key processes that are very poorly understood: the initiation of inflammation, the progression from inflammation to osteoproliferation, and the maintenance of excessive bone formation.

MRI and histopathological studies have provided some insights into these processes in AS patients but the lack of informative samples and difficulties in undertaking large scale longitudinal human imaging studies have limited our knowledge. Mouse studies enable longitudinal and interventionist studies to be undertaken. A key outcome is the dissection of the relationship between inflammation and bone formation and where in the process therapeutic intervention might be most beneficial. However, a number of mouse models exist with differing phenotypes making interpretation and achieving a consensus difficult.

This presentation will present the current understanding of the disease process in humans and summarise the contribution of studies in animal models that have added to our elucidation of AS disease progression and identified potential new therapeutic approaches.
PODIUM 2
INTRODUCTION
Finite element models (FEMs) offer a non-invasive approach to studying cartilage loading during physiological activity. Isotropic hyperelastic models have been previously used to describe cartilage in full-joint FEMs [1]. Despite their prior use, the capability of these models to predict contact variables (i.e., contact force, pressure and area) has not been quantified using direct time-dependent measurements. The aim of this study was to evaluate the limits by which simple isotropic hyperelastic models may describe contact variables for osteochondral plugs in unconfined compression. These limits would indicate the highest accuracies achievable in full-joint FEMs if other modelling features were optimized such as the material constants, geometry and boundary conditions.

METHODS
Forty osteochondral (cartilage + bone) cylindrical plugs (diam. 3 mm) were harvested from the tibiofemoral joint of three cadavers (age 59.1±7.2 years). Arthroscopic examination confirmed that each knee was free of macroscopic defects. Each specimen was tested in unconfined compression using a custom-built electro-mechanical loading apparatus. The bone end of the plug was fixed to a loading platen and the articular surface was pressed against a glass plate lubricated with saline. Axial force and displacement were measured with a load cell and linear variable differential transducer, respectively. Axial loading was applied at a rate of 20% s⁻¹ up to a compressive strain of 30%, which is representative of cartilage compression incurred during walking [2]. A rotation table and camera positioned transverse to the cartilage were used to acquire cartilage geometry prior to testing.

Using the measured specimen geometry, specimen-specific finite element models were created. Cartilage was assumed to be incompressible and was represented using either the neo-Hookean model, characterized by the shear modulus (µ), or the Yeoh model, characterized by two material constants (C₁₀ and C₂₀). These material constants were determined by solving an iterative optimization problem that minimised the root mean square error between the experimental and simulated contact force. The contact force, average contact pressure and contact area obtained from the simulations were compared against their respective values determined experimentally.

RESULTS AND DISCUSSION
The neo-Hookean model over-predicted the experimental contact force at low strain and under-predicted this quantity at high strain (Fig. 1). By comparison, the Yeoh model accurately reproduced the mean contact force at all strains. Both models under-predicted the mean contact area at all axial strains. RMS errors in contact force and average contact pressure were significantly lower (p<0.001) for the Yeoh model compared to the neo-Hookean model (Table 1). RMS errors for contact area were not significantly different (p>0.05) between each model.

CONCLUSIONS
The Yeoh model with a greater number of material constants had a greater capability to describe contact force and pressure compared to the neo-Hookean model. Each model under-predicted contact area. These results suggest that contact variables may be better predicted using an inhomogeneous material with a reduced stiffness at the surface. The RMS errors in the contact variables highlight the limits by which simple isotropic hyperelastic materials may describe measurements of contact force, pressure, area.

REFERENCES
INTRODUCTION
Fractures of the tibial plateau (TPF) are frequently managed with open reduction and internal fixation. However, a number of factors, both surgical, rehabilitation-based, and other lifestyle choices may disrupt the reduction of the articular surface resulting in post-traumatic osteoarthritis. The aim of this study was to investigate if the fracture construct loads are associated with the migration of the fracture fragments over time. We hypothesized that the fracture loading during walking was not sufficient to exceed the elastic limit of the fracture construct and would therefore not result in clinically excessive fracture migration over time.

METHODS
Nine patients with a variety of partial articular TPF patterns were recruited at their time of admission. All patients were treated with ORIF following a standardised preoperative planning intended to achieve fracture specific fixation. All patients were assessed with radiostereometric analysis (RSA) [3] and gait analysis at 2, 12, 26 and 52 weeks following their surgery. Kinematic and kinetic data were collected for each patient walking at a self-selected velocity. At weeks 2 and 12, patients used walking aids as required by pain and balance. Data were recorded using a 12-camera Vicon MX-F20 system (Vicon, Oxford, UK) and two AMTI force platforms (BP400600) at 100 Hz and 400 Hz respectively. From these data muscle forces were computed using a musculoskeletal model based on the geometry of Delp et al. [4] which was fitted to the patient’s anthropometry using uniform scaling. Muscle forces were computed by solving a static optimisation problem minimizing the sum of the squared muscle stresses. All computations were performed in Matlab (The Mathworks, Inc.). From these data the knee joint reaction force (JRF) (a proxy measure of fracture loading) was calculated. To estimate whether the knee was loaded predominantly laterally or medially, we used a method similar to Winby et al. [5] and Gerus et al. [6]. When the point of application of the JRF was < 5 mm from the knee joint centre (KJC) on the mediolateral axis, loading was classified as neutral. When the point of application was > 5 mm from the KJC, the loading was classified as medial or lateral.

To investigate if within patient fracture loading was associated with fracture displacement, a generalized least squares fixed effect model was used at all four assessments on the peak and mean knee JRF during stance phase of gait and fracture migration from RSA.

RESULTS AND DISCUSSION
No significant association was identified between the peak knee JRF during loading response and fracture fragment migration (p=0.274). However, a moderate positive association was identified between mean knee JRF during stance and fracture fragment migration ($R^2 = 0.36, p = 0.014$) (Figure 1). The location of the JRF appeared to change with time based. At week 2 and 12 the patients, on average, loaded their fracture in a neutral manner. At week 26 and 52, the patients, on average, had shifted the load to the medial compartment of the tibia. The same medial shift in the point of application was noted in all fractures.

CONCLUSIONS
The peak load experienced during walking was not associated with fracture migration. However, the average load was, which may be important in fracture stability and healing.

REFERENCES
RESULTS

are calibrated to reproduce rates of bone loss and recovery in rates of these hormonal changes are calibrated to reproduce realistic biochemical coupling between osteoclasts and osteoblasts.

An initial state of bone remodeling is first assumed, in which the tissue across the midshaft cross section remodels at site-specific turnover rates without changing its porosity. OP is then simulated by hormonal changes deregulating the biochemical coupling between osteoclasts and osteoblasts. These hormonal changes are calibrated to reproduce realistic rates of overall bone loss. The strengths of the resorptive and formative responses of bone cells to mechanical regulations are calibrated to reproduce rates of bone loss and recovery in cosmonauts undertaking long-duration space flight missions.

RESULTS AND DISCUSSION

Bone volume fraction distribution over the midshaft cross-section is shown after 40 years of simulated OP (Fig 1a). Highly porous bone (red ring) developed in the endocortical region. Compact bone was preserved in intracortical and periosteal regions (dark blue), but not along the antero-posterior axis (light blue). In Figure 1b, a microradiograph of an 89 yo individual is shown. The same patterns of porosity distributions can be observed. Porous intracortical bone is found near the endosteal surface and in the antero-posterior axis (white arrows), which corresponds to the bending axis.

Both geometrical and mechanical regulations induced site-specific changes. Changes induced by mechanical regulation strongly depend on the distance from the bending neutral axis and resulted in an asymmetry in porosity distribution between the media-lateral and antero-posterior axes. Changes induced by geometrical regulation depend on porosity and led to pronounced loss of bone in the endocortical region. Our main finding is that endocortical bone loss was found to develop mainly due to geometrical regulation, with minimal influence of mechanical feedback.

Figure 1: (a) Bone volume fraction distribution in the femur midshaft cross-section after 40 years of simulated OP. (b) Microradiograph of a midshaft femur of an 89yo individual.

CONCLUSIONS

We conclude that: (i) endocortical bone loss during OP is mainly driven by the influence of microstructure geometry on turnover rate; and (ii) mechanical regulation increases bone loss around the neutral axis, and helps preserve cortical bone near the periosteum, away from the bending axis.

REFERENCES

INTRODUCTION
Musculoskeletal muscle and joint contact forces during activity are used in research and clinical contexts including prosthesis design and injury prevention [1]. Most often, a generic musculoskeletal model is scaled to participant anatomy using corresponding measurements of segment length. However, it is unclear how scaled-generic knee kinematic errors affect muscle and joint contact force calculation. Recently, subject-specific knee kinematics were obtained from patient anatomy using a spatial parallel mechanism [2] while segment lengths, motion data and bone geometry can be obtained using gait analysis and computed-tomography (CT). We assessed the sensitivity of musculoskeletal models to scaled-generic knee kinematic errors by combining subject-specific knee kinematic modelling [2] with earlier motion data and CT images [1].

METHODS
Knee CT images and motion capture data for 13 healthy women ages 60–78 years was taken from a previous study [1]. Reflective markers were attached to the subjects in accordance with Dorn et al. (2012) [3]. Marker trajectories (VICON, Oxford Metrics Group, Oxford, UK) and ground reaction forces (AMTI, Watertown, MA) were recorded during walking, jumping, stair ascent, stair descent, chair sitting, fast walking and a static trial. CT images (voxel size: 0.5 mm) of the knee were obtained via a clinical whole-body scanner (Aquilon CT, Toshiba Corporation, Tokyo). Scaled-generic musculoskeletal models were built by scaling a generic model [3] to individual segmental lengths calculated from the static trial. Dominant leg knee bone geometry was extracted from the CT images using dedicated software (ScanIP, Simpleware Ltd., Exeter, UK). Reference ligament attachment locations and cartilage surfaces were taken from [2] and fitted to the subject knee bone. The subject-specific knee kinematics were estimated using a spatial parallel mechanism that maximized joint congruence at each flexion angle following a published procedure [2]. As a preliminary step, subject-specific model kinematics were visually compared with the literature [4]. Scaled-generic and subject-specific joint angles and moments were calculated by inverse kinematics and dynamics algorithms. Muscle forces were calculated by minimizing the sum of squared muscle activations. Joint contact forces were calculated by solving segment equilibrium. Scaled-generic and subject-specific output were compared by accumulating all data and calculating inter-model coefficients of determination, Root Mean Square Error (RMSE) and peak error (PERR). RMSE and PERR were also calculated with respect to corresponding subject-specific peaks (RMSE% and PERR%).

RESULTS AND DISCUSSION
Four of the 13 subjects have been analysed. There was a good visual agreement between the subject-specific pattern of the knee helical axis and published measurements [4]. Scaled-generic vs subject-specific coefficients of determination for joint angle and joint moment, muscle and joint contact force magnitudes were between 0.96 and 0.99. The joint angle, moment, muscle and joint contact force RMSE’s (RMSE%) were 3.8° (3.8%), 3.1 Nm (3.2%), 32.6 N (1.0%) and 107.6 N (1.9%), respectively. The joint angle, moment, muscle and joint contact force PERR’s (PERR%) were 14.3° (14.5%), 32.6 Nm (33%), 1710 N (53.3%), 1919 N (33.4%) respectively (Fig. 1).

Figure 1: Scaled-generic vs subject-specific joint forces.

CONCLUSIONS
Preliminary results show that scaled-generic knee kinematics are viable in calculating average patterns of musculoskeletal forces while more accurate subject-specific procedures are recommended for individualized modelling.

ACKNOWLEDGEMENTS
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The influence of an assistive robotic exoskeleton on upper-limb muscle and joint function

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INTRODUCTION
Assistive upper-limb exoskeletons may complement physiotherapy of the upper-limb and result in more efficient motor relearning and joint mobility for individuals with severe neuromuscular impairment [1]. However, their capacity to augment muscle and joint function during rehabilitation tasks is not well understood. Since non-invasive force measurement strategies are not available, computational simulations are widely used to infer muscle and joint function. The objective of this study was to develop a three-dimensional musculoskeletal model of the upper limb and to use this to quantify the influence of a robotic exoskeleton on muscle and joint function during simulated weightless motion.

METHODS
Two healthy male adults, P1 (age 35 yrs, weight 82 kg) and P2 (age 24 yrs, weight 77 kg) were seated and interfaced with a 6 degree-of-freedom robotic upper-limb exoskeleton (ArmeoPower, Hocoma, Switzerland) (Fig 1A). The shoulder, elbow and wrist joint centres on the robotic exoskeleton were aligned with each subject’s anatomical joint coordinates. Subjects were asked to perform two upper-limb tasks at each subject’s self-selected speeds, with each task each synchronised to a metronome. The first task was reaching from an initial position (45° shoulder abduction, 0° elbow flexion) to an extended reach position (90° shoulder flexion, 0° elbow flexion); while the second task was touching one’s nose form the same initial position. Each task was performed under two conditions: (1) full-weight-compensation, i.e., the exoskeleton compensated for the entire mass of the subject’s upper extremity and its own weight and inertia; (2) zero-weight-compensation, i.e., with the robotic exoskeleton only compensated for its own weight and inertia.

A musculoskeletal model describing 9 degrees of freedom of motion at the clavicle, scapula, humerus, radius and ulna was developed (Fig. 1B) (OpenSim, SimTK). The model was actuated by 35 Hill-type muscle-tendon units representing the major upper-limb muscle groups. Anatomical joint torques at the shoulder and elbow during the upper-limb motions were calculated with the musculoskeletal model using inverse dynamics. Measured torques applied by the robotic exoskeleton to the upper limb were subtracted from the anatomical torques to obtain the net joint moments. These net joint moments were then decomposed into muscle and joint-contact forces with the model using static optimisation.

RESULTS AND DISCUSSION
Compared to the zero-weight-compensation tasks, the net internal shoulder elevation torques were 68% lower under gravity compensation using the robotic exoskeleton (Fig. 2). This led to smaller muscle forces, including an average 81% smaller middle deltoid force (Table 1), as well as a 43% smaller glenohumeral joint force magnitude. Counter-intuitively, the net elbow flexion moment increased by 51% during gravity compensation tasks, most likely due to the subjects over-exerting against the exoskeleton.

| Table 1. Averaged middle deltoid (DEL), infraspinatus (INFRA), subscapularis (SUBS) and glenohumeral joint (GHJ) force magnitude during full-weight-compensation (assisted) and zero-weight-compensation (unassisted) motion with the upper-limb exoskeleton (units are Body Weight) |
|---|---|---|---|
| | Unassisted | Assisted |
| DEL | 0.16 | 0.03 |
| INFRA | 0.20 | 0.10 |
| SUBS | 0.07 | 0.03 |
| GHJ | 0.37 | 0.21 |

CONCLUSIONS
This study demonstrates that an upper-limb robotic exoskeleton has the potential to significantly reduce shoulder muscle and joint-contact forces. Increases in joint moments may occur when subjects exert against the exoskeleton (resistive movements). The quantitative modelling framework in this study may be useful for targeted intervention in stroke patients using robotic assistive rehabilitation technology. Future studies will employ electromyography-informed methods to derive muscle excitations.

REFERENCES

Figure 1: The ArmeoPower robotic assistive rehabilitation exoskeleton (A) and a posterior view of the musculoskeletal upper extremity model (B)
ORAL POSTERS 2
MANUFACTURE OF HUMAN ENGINEERED TENDON IN AN EX-VIVO BIOREACTOR SYSTEM


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INTRODUCTION
Harvesting of donor tendon and ligament tissue for tendon reconstructive surgery is always an issue. The objective of this study is to examine feasibility of construction of scaffold-free engineered tendon under mechanical stimulation in a bioreactor system using autologous tendon progenitor cell (TPC) from a needle biopsy of tendon tissue.

METHODS
TPCs were isolated from mice and from needle biopsy of human patella tendon. They were then characterized by colony forming, flow cytometry and differentiation assay. TPC were treated by connective tissue growth factor (CTGF) and ascorbic acid in culture before placing in a bioreactor. They were then cultured either in loading-free environment or under mechanical stimulation (6% tensile strain at 0.25 Hz, 8h/day) in a bioreactor system for 7 days. Histology, immunohistochemistry, qRT-PCR and mechanical test were performed to characterize the engineered tendon.

RESULTS
After the validation of various conditions, we have manufactured neo tendon tissue that exhibited well organized collagen structure with elongated cell morphology. Immunohistochemistry showed the expression of type I collagen and tendenmodulin of the tissue. Molecular assessment showed neotendon tissue express tendon matrix molecules and specific tendon transcription factors necessary for tendon maturation. Furthermore, mechanical stimulation is able to induce tenogenic differentiation of TPCs evidenced by increased expression of tenogenic marker and decreased expression of adipogenic, osteogenic and chondrogenic markers at both gene level and protein level. Lastly, subjected to cyclic tensile loading, the neo-tendon tissue showed acceptable mechanical properties as compared to the control and some contained necrotic bone fragments. Bone surrounding cysts was sclerotic.

CONCLUSIONS
We showed that our bioreactor is able to guide differentiation of TPC into neo-tendon tissue. Autologous tendon cells from biopsy could generate neo-tendon tissue in bioreactor system, which hold potentially promising for tendon reconstruction.
THE INTERACTIONS BETWEEN INFLAMMATORY CYTOKINES AND FIBRIN CLOT PROPERTIES

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INTRODUCTION
The early inflammatory response characterized by pro-inflammatory cytokines plays a chief role in the process of bone fracture healing following trauma. In our previous studies we found noticeable differences in the levels of interleukin-1 beta (IL-1β) between hematomas formed in the smaller compared to the larger bone defects. Yet, this knowledge that IL-1β could influence hematoma structures is still vague.

METHODS
The effects of IL-1β on fibrin polymerization process were investigated using turbidity measurements at different concentrations (0, 50, and 500 pg/mL). We then assessed, the morphological differences in fibrin formation of clots using confocal microscopy (CM). The rigidity of clots was analyzed by compaction studies to evaluate the mechanical integrity. The clot dissolution (fibrinolysis) process was examined by measuring D-dimer product amounts.

RESULTS AND DISCUSSION
The lag time of protofibril formation was significantly longer in the control group compared to IL-1β groups (Figure 1). In contrast, V_{max} and maximal turbidity were higher in the control group. Under CM observation, the fiber diameters were increased in the control group compared to IL-1β groups. Additionally, the density of fiber formation was thicker in the control group compared to IL-1β groups. Clot rigidity analyzed by compaction study revealed that 500 pg/mL IL-1β can produce clots with thinner and denser fibers, which was consistent with the outcomes from fibrinolytic study showing that the compact fibrin clots contributed to a lower susceptibility to dissolve.

CONCLUSIONS
In summary, we can conclude that IL-1β can considerably reduce fiber diameter and increase fiber density, which may provide a promising target for the treatment of thrombolytic therapy.
INTRODUCTION

Osteoimmunology is an emerging field that studies the interplay between the skeletal system and the immune system. The exploration of this interaction begins with the initial understanding that the skeletal microenvironment facilitates the development of the hematopoietic stem cells (HSCs) and mesenchymal stromal cells (MSCs), from which all immune cells and bone cells derive, and that various immunoregulatory cytokines affect the fate of bone cells.

One of the important cytokines secreted by MSCs is the vascular endothelial growth factor (VEGF), the expression of which increases during osteogenic differentiation. Previous study has found that VEGF is involved in the secretion of certain pro-inflammatory cytokines and may participate in paracrine mechanisms of vascular remodelling \[1\]. Inhibition of VEGF can control tumour growth and the infiltration of suppressive immune cells \[2\]. However, the immunoregulatory properties of VEGF secreted from MSCs during osteogenesis are still elusive, leaving a certain gap unfilled in osteoimmunology. The aim of the present study was to investigate the secretion changes of VEGF during MSC differentiation and its role in immunoregulation.

METHODS

Human MSCs were isolated and induced with osteogenic medium. The conditioned medium were collected from Day 0 (MSC-CM) to Day 14 (OMSC-CM) (Figure 1). An antibody-based cytokine array was applied to identify the cytokines in MSC-CM and OMSC-CM.

Lipopolysaccharide (LPS) was added to OMSC-CM to stimulate macrophage-like cell line RAW264.7 for 12 hours. The expression of inflammatory cytokines such as IL-1β, IL-6 and TNFα was investigated by RT-qPCR and ELISA. The expression of CCR7 was determined by fluorescence-activated cell sorting (FACS).

To further demonstrate the immunoregulatory effect of VEGF secreted from MSCs, neutralizing antibody targeting VEGF was added to the OCM. Additional experiments were performed using various concentrations of recombinant VEGF to stimulate RAW264.7 cells. The expression of pro-inflammatory cytokines was again monitored by RT-qPCR and ELISA.

RESULTS AND DISCUSSION

The result from the cytokine array revealed that 18 genes were up-regulated and 4 were down-regulated during osteogenic differentiation, among which the secretion of VEGF increased dramatically. Our indirect co-culture study demonstrated that the expression of pro-inflammatory cytokines (IL-1β and IL-6) in macrophages was induced by the stimulation of OMSC-CM. Furthermore, the addition of VEGF neutralizing antibodies could modify the pro-inflammatory effect of osteogenically differentiated MSCs.

CONCLUSIONS

Our results showed that the secretion of VEGF by MSCs was up-regulated during osteogenic differentiation, which enhanced the pro-inflammatory cytokine expression in macrophages. The secreted VEGF may play an important role in the immunoregulatory shift during MSC osteogenic differentiation.

REFERENCES

A NEW METHOD FOR STUDYING THE PHENOTYPIC SHIFT OF MACROPHAGES DURING BONE REMODELING

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INTRODUCTION

Macrophages derived from hemopoietic progenitors and circulating monocytes play an important role in bone homeostasis and regeneration [1]. Classically activated macrophages (M1) are developed in response to interferon-gamma (IFN-γ) and microbial stimuli such as lipopolysaccharide (LPS), which activate the toll-like receptor-4 (TLR-4) signalling. Alternatively, activated macrophages (M2) in vitro are controlled by interleukin-4 (IL-4) and interleukin-13 (IL-13) [2].

The majority of the studies on macrophages have been developed in the context of host defense. Therefore, the traditional approach to study macrophages is normally based on pathogen stimulation. However, macrophages are found indispensible in the process of bone remodeling during which macrophages are rarely activated by pathogens [3]. These observations implicate that macrophages may be more multifunctional than originally appreciated, with different physiologies and distinct immunological functions.

This study aims to investigate the immunoregulatory effect of exogenous factors on the phenotypic shift of macrophages under physiological rather than pathological conditions and to establish a novel approach for the study of macrophage during bone remodeling.

METHODS

A murine macrophage cell line RAW264.7 was cultured in normal growth medium supplemented with vascular endothelial growth factor-A (VEGF-A) and bone morphogenetic protein-2 (BMP-2) as the exogenous stimuli. Proinflammatory cytokines secreted by macrophages was examined by RT-PCR and ELISA. The morphological changes of macrophages were examined by phalloidin staining.

The M1 phenotype of RAW264.7 cells was activated by LPS and IFN-γ and the M2 phenotype was activated by IL-4 respectively. VEGF and BMP-2 were added to M1 and M2 cell culture medium. M1 and M2 related genes were analyzed by RT-PCR. The expression of inducible nitric oxide synthase (iNOS) and arginase were examined by immunofluorescence staining.

RESULTS AND DISCUSSION

Morphological changes were observed in macrophages induced with VEGF and BMP-2 with/without LPS+IFN-γ or IL-4 stimulation (Figure 1). Our data has demonstrated that M1 markers such as iNOS, C-C chemokine receptor type 7 (CCR7), cluster of differentiation 86 (CD86) and integrin alpha X (ITGAX, CD11c) were up-regulated by VEGF and down-regulated by BMP-2 with/without supplementation of LPS and IFN-γ. M2 markers such as Arginase, interleukin-10 (IL-10), cluster of differentiation 163 (CD163), cluster of differentiation 206 (CD206) were up-regulated by BMP-2 and down-regulated by VEGF with/without IL-4 stimulation.

CONCLUSIONS

Our results indicate that exogenous stimuli were able to activate macrophage polarization without the precondition of LPS+IFN-γ or IL-4, which is more relevant to the physiological bone homeostasis. The interplay between exogenous factors and the phenotype shift of macrophages under non-inflammatory conditions should be elucidated when evaluating the in vitro osteogenic capacity of target proteins and biomaterials (Figure 2).
Co-culture system to study the interactions between notochordal and mature nucleus pulposus cells exposed to hydrostatic pressure.

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INTRODUCTION

The nucleus pulposus of the intervertebral disc (IVD) contains two distinct cell types, notochordal cells (NC) and mature nucleus pulposus cells (MNP)1. The ratio of these cell types varies with species, age and health, with higher proportions of NC cells being associated with proteoglycan-rich healthy NP tissue, while MNP cells associated with more fibrous tissue which is prone to structural failure1. Co-culture studies have found that NC cells stimulate proteoglycan production in MNP cells2. Additionally NC cell-conditioned media has been shown to stimulate proteoglycan production in MNP cells and protect them from inflammatory induced apoptosis3. Our previous work found that MNP cells are sensitive to mechanical stress while NC cells are not. While other studies have focused on the protective properties of NC cell-conditioned media, few studies have investigated the bidirectional interactions between these cells. Additionally, no studies have looked at the effect of co-culture under mechanical stress (hydrostatic pressure). Traditional co-culture inserts use polycarbonate or polyethylene terephthalate (PET) membranes in plastic inserts within tissue culture plates. However, hard plastic tissue culture plates cannot be used inside a hydrostatic pressure vessel. This study aims to develop a co-culture system that is compatible with hydrostatic pressure experiments in order to test the hypothesis that NC cells can protect MNP cells from mechanical stress. Large pore size (0.4µm) membrane divided soft plastic pouches will allow NC and MNP cells to be kept physical separate but share the same media allowing signaling between the cell types.

METHODS

A 3D in vitro model will be used to culture isolated bovine caudal MNP and NC cells seeded in alginate beads at physiological ratios. Beads are cultured in media-filled soft plastic pouches to ensure equal distribution of pressure to cells (Fig 1A). Pouches will be divided into two compartments, separated by a semi-permeable membrane (Fig. 1A, B). Cells will be exposed to pathological levels (1.6-2.4 MPa) of hydrostatic pressure using a custom-built pressure vessel for up to 24 hours (Fig. 1C). Following pressurization, cells will assayed for viability, proteoglycan production and ECM gene expression. Media will be assessed for growth factors and cytokines.

RESULTS and DISCUSSION

NC cells represented 8% of the total cell population in nucleus pulposus yet produced 20 times more GAG than MNP cells.

CONCLUSIONS

This study presents a novel cell culture system to study the cells of the nucleus pulposus under physiologically relevant mechanical stress.

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REFERENCES

Preparation of Magnesium-Containing Biocompatible MAO Coatings on Ti6Al4V Alloy

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INTRODUCTION
Micro-arc oxidation (MAO) is an important surface modification technology, which can produce porous, relatively rough and firmly adherent oxide ceramic coatings on the titanium alloy surface. According to the structure of natural bone tissue with organic and inorganic components, in this study we design a biocompatible magnesium-containing composite coating, which is formed via MAO in the electrolyte containing phytic acid and EDTA-MgNa.

METHODS
The Ti6Al4V samples were anodized at 50mA/cm² in an aqueous solution containing 15 g/l phytic acid and different concentrations of EDTA-MgNa (5 g/l, 10 g/l, 15 g/l, 20 g/l) (denoted as S5, S10, S15, S20). MC3T3-E1 preosteoblasts were cultured on various sample surfaces to examine cytocompatibility.

RESULTS AND DISCUSSION
Fig.1 shows surface morphology of MAO coatings formed in different concentrations of EDTA-MgNa. As shown, the S5 and S10 samples display porous structure with the pore size from 0.1 to 2.0 μm, in comparison the S15 and S20 samples reveal more homogeneous porous structure with the pore size from 1.0 to 3.0 μm. Mg content of the oxide film is gradually increased with the enhancement of EDTA-MgNa concentration in electrolyte. It illustrate that the EDTA-MgNa concentration can regulate the surface composition, morphology as well as the pore size of the titanium oxide film. High concentration of EDTA-MgNa is favorable to increase pore size and Mg content of oxide film.

Fig.2 reveals preosteoblasts proliferation and differentiation on various MAO samples. Gradual increases in the cell proliferations are observed on all sample surfaces throughout the culturing period. In particular, S15 sample show the highest cell proliferation on day1 while sample S5 show the highest cell proliferation on days 3 and 7 compared with other treated samples. In addition, significant increased ALP activity levels can be observed on S5 and S15 after 14 and 21 days of culture. Generally, S15 and S5 samples present better cell proliferation and differentiation than other samples, whereas S20 sample shows the worst cytocompatibility. Our data indicate minute amount magnesium ions effectively promote preosteoblasts proliferation and differentiation, but when magnesium ion content in the MAO coatings increase to around 2.3wt%, negative effect on cytocompatibility occur.

CONCLUSIONS
Magnesium-containing biocompatible ceramic coatings can be formed on Ti6Al4V alloy via MAO. High concentration of EDTA-MgNa in the electroly is favorable to increase pore size and Mg content of oxide film. Minute amount Mg in the oxide film effectively promotes preosteoblasts proliferation and differentiation, but negative effect on cytocompatibility is produced when Mg content is increased to 2.3wt%. Our study suggests biocompatible magnesium-containing titanium alloys have attractive potential as orthopedic implants.

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REFERENCES
FEASIBLE EXPANSION SIZE OF A NOVEL EXPANDABLE FASTENER IN THE CERVICAL SPINE

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INTRODUCTION
Studies [1,2] have reported the potential for expandable fasteners to provide increased pull-out strength compared to conventional screws. The author’s previous work on a novel expandable fastener for cervical spine fixation demonstrated a 41% increase in pull-out strength against an equivalent sized orthopaedic screw [3]. This study investigates the maximum potential expansion size and expandable fastener length along common fastener trajectories in the cervical lateral mass and vertebral body.

METHODS
8 human C5 vertebrae and a single cervical spine (C3-C7) (n=13) were scanned using the Skyscan 1176 micro-CT scanner at the Centre for Microscopy, Characterisation and analysis at the University of Western Australia with 18 microns resolution and a rotation step of 0.5°. A cylindrical volume of interest was digitally created for each fastener trajectory in each sample based on modified clinical screw techniques. The techniques that were assessed were the Magerl and Lee lateral mass (LM) approaches and a conventional, parallel single and dual fastener anterior cervical vertebral body (VB) approach. The samples were cortically segmented using a modified Buie method [4]. The maximum expansion diameter at a given fastener depth was defined as the largest diameter circle that could fit without including any cortical bone (see fig. 1). This methodology assumes that the fastener can expand right to the cortex, providing an upper limit to expansion size.

RESULTS AND DISCUSSION
As can be seen in figure 2, in the lateral mass, the modified LM Roy-Camille technique allows high expansion only for a limited fastener depth whereas the modified LM Lee method provides only limited opportunity for expansion at a significant fastener depth. In the vertebral body, significant expansion size is available close to the maximum fastener depth for both the VB single and dual fastener approach. The VB single fastener approach does not significantly increase expansion size (p=0.762) but increases fastener depth by 2.2 mm on average (p=2.99E-06).

CONCLUSIONS
The cervical lateral mass has limited potential for significant expansion at significant depth and furthermore the maximum expansion depth is inconsistent, increasing the risk of cortical cracking. However, the cervical vertebral body has significant potential for a large expansion size at close to maximum depth and is therefore suited to expandable fasteners with a large expansion size. The data from this study will used to select dimensions for future expandable fastener prototypes which will be empirically tested in human cadaveric vertebral bodies to ensure cortical cracking does not occur.

REFERENCES
PODIUM 3
Inter-Transverse Process Spinal Fusion Induced by Electrical Stimulation to Dorsal Root Ganglion

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INTRODUCTION
Periosteum, endosteum and bone are innervated by sensory nerves expressing calcitonin-gene-related peptide (CGRP) which is a known osteoanabolic peptide and plays an important role in fracture healing and spinal fusion [1]. Synthesis and release of CGRP are found in sensory neurons located in dorsal root ganglions (DRG) and can be upregulated by electrical stimulation at DRG [2].

The present study was to test a concept of electrical stimulation at dorsal root ganglion (DRG) via an implantable micro-electrical stimulation system (IMESS) to achieve an inter-transverse process fusion without bone grafting in rat model.

METHODS
A novel IMESS was developed [3] for stimulating L4-L6 DRG in rats. The IMESS consists of 3 parts: a body consisted of a circuit board and a button-type battery, 3 electrodes, and wires connecting the body and the electrodes (Figure 1). Sixteen rats were used and divided equally into the control group without electrical stimulation and the electrical stimulation (ES) group with a daily 20 minutes electrical stimulation to DRG for 6 weeks. At the end of 6 weeks, radiography and micro-computed tomography (micro-CT) were conducted to evaluate new bone formation and spinal fusion. Bilateral L4-L6 DRGs were harvested for immunohistochemistry and quantification of neurons with upregulated CGRP-expression.

RESULTS AND DISCUSSION
In the group ES, rate of radiographic fusion (Figure 2A) with complete and uninterrupted bony bridging was 100% (8/8) at the right L4/L5 transverse processes and 75% (6/8) at the right L5/L6 transverse processes. Bony callus formation was absent at the left L4-L6 transverse processes in the group ES, as well as in bilateral L4-L6 transverse processes in the control group. Bony fusion was proved with 3D reconstruction of micro-CT image (Figure 2B). CGRP-expressing neurons in DRG were visualized by immunohistochemistry. By comparing the CGRP signals in the right DRG between ES and control groups, enhanced CGRP signal was observed only in group ES, but not in non-stimulated control group (Figure 3).

Figure 1. Anterior-posterior view (A) and lateral view (B, C) of IMESS implanted in rat back.

Figure 2. X-ray anterior-posterior view of intertransverse spinal fusion (A) and micro-CT 3D reconstruction.

Figure 3. DRG immunohistochemistry. Enhanced expression CGRP only in ES group.
INTRODUCTION
The local mechanical environment plays an important role in the pathogenesis of knee osteoarthritis (OA) [1]. Knee joint loading, estimated using surrogate measures such as the external knee adduction moment (EKAM), has been implicated in the development of knee pain and radiographic progression of medial knee OA in older adults [2]. Lateral wedge insoles (LWI) are intended to reduce biomechanical risk factors of medial knee OA progression, such as increased knee joint load; however, there has been no definitive consensus on this topic. The aim of this systematic review and meta-analysis was to establish the effects of lateral wedge insoles on knee joint load in people with medial knee OA.

METHODS
Six databases were searched from inception until February 13th 2015. Eligible studies reported on the biomechanical effects of LWIs in people with medial knee OA during walking. Primary outcomes of interest relating to the biomechanical risk of disease progression were the 1st and 2nd peak EKAM and knee adduction angular impulse (KAAI). Point estimates of effects were extracted and cross-checked by two reviewers. Standardised mean differences were calculated (Hedges g: SMD). Where not reported, the standard error of the mean difference and correlations between outcomes were estimated from P-values using the equivalent T-statistic. When this was not possible, an imputation approach was taken [3]. Meta-analysis was performed in Review Manager software (v5.2, Cochrane Collaboration, Oxford, UK) using the inverse variance method and a random-effects model. Statistical heterogeneity was assessed using the I² statistic.

RESULTS AND DISCUSSION
Three hundred and eighty three records were identified. After assessing eligibility against the criteria, 48 studies were retained for full-text screening. Eighteen studies with a total of 524 participants met all eligibility criteria and were included in the final review. All studies included radiographic assessment of knee OA, most commonly defined as ≥ K-L 2. Medial joint space narrowing greater than lateral and/or varus knee alignment were used to limit participants to those with medial knee OA. Most studies investigated full-length insoles (n=14), typically with an insole inclination angle of five degrees, with insole angles ranging from four to 11 degrees.

Lateral wedge insoles resulted in a small but statistically significant reduction in the 1st peak EKAM (SMD: -0.19; 95% CI -0.23 – -0.15) and 2nd peak EKAM (SMD: -0.25; 95% CI -0.32 – -0.19) with a low level of heterogeneity (I² = 5% and 30%, respectively). This represents a small effect size and equates to an absolute change in the 1st peak EKAM of approximately 0.15 %BW*Ht. There was a small reduction in the KAAI with LWIs (SMD: -0.14; 95% CI -0.21 – -0.07, I² 31%). The pooled effect size equates to an absolute change in the KAAI of approximately 0.05 Nms/BW*Ht.

CONCLUSIONS
Lateral wedge insoles cause consistent but modest reductions in biomechanical risk factors for knee OA progression. The magnitude of changes in surrogate measures for knee loading make it unclear whether they have the potential to have a structurally modifying effect. Moreover, limitations in inferring alterations in knee joint contact force from external moments should be considered. Due to high variability in individual response, interrogation of their potential to attenuate structural changes in biomechanical phenotypes associated with larger reductions in knee load is warranted.

REFERENCES
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   Richard Jones may receive royalties from Salford Insole™, a manufacturer of lateral wedge insoles.
Low Wear of Highly Cross-Linked Polyethylene in Total Hip Replacement at Five Years

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INTRODUCTION
Highly cross-linked polyethylene (XLPE) liners are manufactured using a variety of methods that result in different amounts of cross-linking which may influence in vivo wear properties. The improved wear properties of XLPE have encouraged the use of larger diameter articulations to address the most common cause of early revision surgery, dislocation.

The aim of this study was to measure the wear rate of XLPE using radiostereometric analysis (RSA) and investigate the influence of: 1) XLPE design; 2) articulation size; and 3) patient age.

METHODS
Patient Cohorts
Six patient cohorts including a total of 157 hips were prospectively enrolled into clinical wear studies at the Royal Adelaide Hospital, Wakefield Hospital and Repatriation Hospital in Adelaide, South Australia (Table 1). These cohorts differed with respect to design of XLPE, articulation size and patient age. Cohorts B and C were two arms of a randomised controlled trial investigating the effect of articulation size on wear.

Table 1: Patient Cohorts

<table>
<thead>
<tr>
<th>#</th>
<th>XLPE TYPE</th>
<th>HEAD SIZE</th>
<th>AGE RANGE</th>
<th>Number Recruited</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5Mrad</td>
<td>28</td>
<td>55-80</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>10Mrad</td>
<td>28</td>
<td>65-75</td>
<td>27</td>
</tr>
<tr>
<td>C</td>
<td>10Mrad</td>
<td>36</td>
<td>65-75</td>
<td>29</td>
</tr>
<tr>
<td>D</td>
<td>10Mrad</td>
<td>28</td>
<td>40-64</td>
<td>31</td>
</tr>
<tr>
<td>E</td>
<td>3Mrad x3</td>
<td>32</td>
<td>47-76</td>
<td>21</td>
</tr>
<tr>
<td>F</td>
<td>3Mrad x3</td>
<td>36/40</td>
<td>55-76</td>
<td>19</td>
</tr>
</tbody>
</table>

Radiostereometric Analysis
All patients had tantalum markers (1.0mm diameter) inserted within the XLPE liner at the time of surgery. The total femoral head penetration was calculated using the 1 week radiograph as the reference examination. Wear rates were calculated after one year.

RESULTS AND DISCUSSION
The mean proximal bedding-in within the first year was higher for cohort A who received a liner which has a lower dose of irradiation (Figure 1). The mean proximal wear rate was low for each cohort [1,2] and indicated XLPE manufacturing method did not influence the wear rate at five years (Figure 2). There was no difference in the wear rate of 28 and 36mm articulations in cohorts B and C at 3 years.

CONCLUSIONS
This study used RSA to measure the wear of XLPE liners in 157 patients within six different cohorts. This is a significant contribution to the published literature in which only 207 patients have been reported to date, despite the widespread international use of these XLPE liners. The results to date suggest that the wear of XLPE is low. Manufacturing method and design may influence bedding-in within the first year but does not appear to influence wear rate between one and five years. This study indicated that articulation size did not affect XLPE wear between one and five years.

ACKNOWLEDGEMENTS
Research funding was received to conduct this study from NHMRC, Zimmer, Stryker and DePuy.

REFERENCES
Reduced Subsidence with Improved Femoral Impaction Grafting Techniques

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INTRODUCTION

Femoral impaction grafting in revision total hip replacement has potential advantages, including restoration of bone and the need for long stems in young and middle-aged patients. However, early clinical results of femoral impaction grafting in the 1990’s showed stem subsidence of greater than 5mm [1] and postoperative femoral fracture up to 15% [2]. This study aimed to compare the early clinical results and stem subsidence between four consecutive series of revision hip replacement cases with femoral impaction bone grafting to evaluate the effects of developments in technique.

METHODS

Patient Cohorts
In the original series 1 (n=23), bone graft was irradiated at 25kG. In series 2 (n=12) non-irradiated double washed graft was used and long stems were used as required. In series 3 (n=22) modular tamps were used and in series 4 (n=40) a vibrational impactor was used in conjunction with modular tamps (Figure 1). Sensitive radiographic analysis techniques, EBRA and RSA, were used to measure stem subsidence.

RESULTS AND DISCUSSION

At latest follow-up major stem re-revision was required in 5 hips in series 1 (4 for loosening), no hips in series 2 or 3 and one hip in series 4. Minor stem revision, involving cement-within-cement exchange, was undertaken in one hip in series 1 and one in series 2. Periprosthetic fractures occurred in two hips in series 1 (4%), in one hip in series 2 (8%), in one hip in series 3 (4%) and in three hips in series 4 (8%). The three fractures in series 4 all united with internal fixation and the femoral components remained stable.

There was a statistically significant reduction in stem subsidence at the cement-bone interface at 12 months between series 1 and series 2, 3 and 4 (p<0.05). The median stem subsidence of series 1 was 2.1mm at 12 months. In contrast, the subsidence series 2, 3 and 4 were below 1mm similar to that of cemented stems in used in primary total hip replacement [3]. Despite stem subsidence being a major complication of femoral impaction bone grafting very few studies distinguish between stem subsidence at the prosthesis-cement interface from that at the cement-bone interface. Importantly in series 3 and 4 of our study, there was negligible stem subsidence at the cement-bone interface.

CONCLUSIONS

Technique developments in femoral impaction grafting, including the use of modular tamps and a vibrational impactor designed to simplify the procedure, yields excellent early clinical and radiographic results. Using RSA, we have shown that the fixation of the stems in bone is comparable to that achieved in primary hip replacement.

ACKNOWLEDGEMENTS

No research funding was received in relation to the conduct of this study.

REFERENCES


Figure 1: (A) A preoperative AP hip radiograph shows a loose Charnley stem in a 64-year-old man in Series 3. AP radiographs show the same hip (B) postoperatively after IBG and revision to a cemented collarless double taper CPT1 stem and (C) at 3 years postoperatively.

Radiostereometric Analysis
All patients in series 3 and 4 had tantalum markers (1.0mm diameter) inserted within the femoral bone and cement at the time of surgery. The subsidence was calculated using the 1 week radiograph as the reference examination. Subsidence of the femoral stem was measured in relation to the cement and femoral bone separately.
INTRODUCTION
The general recommendation for a failed primary unicompartmental knee arthroplasty (UKA) is revision to a total knee arthroplasty (TKA). Many surgeons prefer to use UKAs in younger patients to postpone TKA believing that the results after revision from a UKA to a TKA is equal to a primary TKA and better than a revision TKA [1-4]. For this to be true the rev-UKA should outperform the rev-TKA. The purpose of the present study was to compare the outcomes, surgical procedures, and mode of failure of failed primary UKAs and primary TKAs revised to TKAs.

METHODS
The study was based on 768 failed primary TKAs revised to TKAs (rev-TKAs) and 578 failed primary UKAs revised to TKAs (rev-UKAs) and reported to the Norwegian Arthroplasty Register (NAR) between 1994 and 2011. Patient-reported outcome measures (PROMs) including the EQ-5D, the Knee Injury and Osteoarthritis Outcome Score (KOOS), and Visual Analogue Scales assessing satisfaction and pain were used. Kaplan-Meier and Cox-regression analyses were performed to assess the survival rate and the risk of re-revision. The independent student’s t-test and multiple linear regression were used to estimate the differences in mean scores in PROMs between the two groups.

RESULTS AND DISCUSSION
Overall, 12% of rev-UKAs and 13% of rev-TKAs were re-revised between 1994 and 2011. The 10 years survival percentage of rev-UKAs vs rev-TKAs was 82 vs 81%, respectively (p=0.1) (Figure 1). There was no difference in the overall risk of re-revision for rev-UKAs vs rev-TKAs (RR=1.3; p=0.1), nor did we find any differences in the PROM scores. However, the risk of re-revision was 2 times higher for rev-TKA patients aged over 70 years (RR= 2.2; p=0.04). Loose tibia (28 vs 17%), pain alone (22 vs 12%), instability (19 vs 19%), and deep infection (16 vs 31%) were major causes of re-revision for rev-UKAs vs rev-TKAs, respectively but the observed differences were not statistically significant. The surgical procedure for rev-TKAs took longer time (mean=150 (SD 52) vs 114 (SD 32) minutes) and more of the operations needed stems (58 vs 19%), stabilization (27 vs 9%), and bone impaction (24 vs 19%) compared to rev-UKAs.

CONCLUSIONS
The outcomes of rev-UKA and rev-TKA in terms of survival, functional outcome, level of pain, patients’ satisfaction, and change in health related quality of life was similar. Similarly, the two revision groups had no significant differences in reasons for re-revision. However, the surgical procedure for rev-TKA seems to be more technically complex compared to rev-UKA. Thus, the argument that the outcome of revising UKA to TKA is similar to primary TKA could not be supported by the present study findings.

REFERENCES
INTRODUCTION
Tibial Plateau Fractures (TPF) are considered to be an independent risk factor for surgical site infection [1]. In an effort to address this problem we have developed an angiosome-sparing anterolateral approach to treat these fractures [2]. In the angiosome-sparing approach the skin perforators are preserved and internal fixation is performed without elevation of the tibialis anterior muscle from the proximal tibia. During the transition phase from the classic anterolateral approach to the angiosome-sparing anterolateral approach, and when the perforators do not allow plate fixation that spares tibialis anterior, a hybrid anterolateral approach was performed. In the hybrid approach, the skin perforators were preserved but the internal fixation involved some degree of tibialis anterior elevation. This study aimed to compare surgical site infection, radiographic, and functional outcomes between patients with TPFs stabilised using the classic, hybrid or angiosome-sparing anterolateral approaches.

METHODS
Between Jan, 2004 & Sept, 2012, 141 TPFs of 138 patients, were treated with a classic anterolateral approach (N=79, Group 1), with a hybrid approach (N=13, Group 2), or an angiosome-sparing approach (N=49, Group 3).

Data including patient and fracture characteristics, treatment methods and clinical and functional outcomes were recorded prospectively and collected retrospectively from electronic medical records, case-notes, and radiographs. Follow-up was for up to 5 years. Categorical, ordinal and continuous variables were compared for the two groups using Chi-squared, Kruskal-Wallis and ANOVA analyses, respectively (alpha 0.05), with appropriate post-hoc analyses.

RESULTS AND DISCUSSION
Patient groups were equivalent (p>0.05) with respect to age (mean 48.3/40.5/46.0 yr), sex (51/11/27 M), smoking (21/5/9), previous deep vein thrombosis or pulmonary embolism (2/0/0) and ASA Physical Status score (1: 39/36/39%; 2: 53/47/46%; 3: 8/17/15%). Fracture characteristics were also equivalent (p>0.05): 56/59/54% were left sided, and 98/100/100% were closed. AO fracture classifications A3, B2 and B3 were distributed similarly between groups, and Schatzker classifications were majority Type II: 60/54/61% and VI: 32/31/31% (p>0.05). Operative duration (3.6/3.4/3.6 hr) was equivalent, as was total (13.7/14.5/13.0 d), pre- (4.0/5.1/4.2 d) and post-operative (9.7/9.4/8.9 d) length of stay (p>0.05). Fewer patients in Group 1 than Group 2 or 3 were allowed partial or a tolerated weight bearing (p<0.05, 29.1/92.3/77.5%), or knee range of motion ≥30° (p<0.05, 49/82/91%) postoperatively.

Postoperative complications were noted for 12(15.2%) patients in Group 1, 0 in Group 2, and 4(8.2%) in Group 3 (p>0.05); and postoperative wound healing was abnormal in 5(6.3%) Group 1, 0 in Group 2, and 1(2%) in Group 3 (p>0.05). For Group 1, 2 and 3, respectively, deep infection occurred in 4(5.1%), 0 and 0 knees, and superficial infection in 2(2.5%), 0, and 1(2%) knees (p>0.05). Further surgical intervention was required for metal removal (4/0/2) and total knee replacement (2/0/0) at statistically equivalent rates (p>0.05).

There was no difference in medium (≤2yr: 63.5/64.8/71.2) and long (>2yr, 67.0/71.3/78.4) term Lysholm scores (p>0.05). There was no statistical difference in the immediate postoperative reduction of tibial plateau width (7.7/3.4/6.3 mm), lateral step (8.6/5.7/7.9 mm) or angulation (0.2/2.7/1.1 deg). There was no significant difference in change in tibial width (0.5/0.5/2.8 mm), lateral step (0.6/-0.7/0.3 mm) or angulation (0.0/0.7/-0.3 deg) at long term follow-up compared with immediate postoperative imaging.

CONCLUSIONS
In this case series of similar patient cohorts, we observed similar clinical, radiographic and functional outcomes for the classic, hybrid, and angiosome-sparing approaches for TPF fixation. However, patients in the latter groups were allowed greater immediate post-operative weight bearing and range of motion, and tended towards fewer postoperative complications, abnormal wound healing and deep infections.

ACKNOWLEDGEMENTS
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REFERENCES
METAL ARTEFACT REDUCTION IN ORTHOPAEDIC IMPLANTS USING MARS SPECTRAL CT

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INTRODUCTION
MARS – Medipix All Resolution System is a multi-energy CT modality that employs photon-counting x-ray detectors developed by the CERN. It has been successfully used in preclinical studies to quantify targeted nanoparticles in cancer cells, characterizing vulnerable atherosclerotic plaques and quantitative imaging of articular cartilage. Our study exploits this novel detector technology for imaging of metal implants. Implant design and characterization relies on imaging modalities such as microCT (µCT) and dual-energy CT. Implant revision relies on radiographic findings. One of the major drawbacks of CT imaging is the metal artefacts around implants that affect image quality and limit clinical diagnosis. This is caused due to a physical process known as ‘beam hardening’ wherein low energy x-rays cannot penetrate dense metal objects thereby causing dense streaks and cupping artefacts. We employed a small-animal MARS-CT to image metal scaffolds and small titanium implants to study reduction in metal artefacts.

METHODS
We used a MARS spectral scanner [1-3] equipped with a Medipix3RX detector with a cadmium telluride sensor. Metal samples made from titanium (Ti6Al4V), cobalt-chromium alloy, stainless steel and magnesium were scanned. Samples were phantoms or actual implants (screw, femoral head and porous scaffolds) with interfacing bone or soft-tissue surrogate. For comparison, some of these samples were also scanned using a commercial µCT scanner. Images were reconstructed using a custom algebraic reconstruction technique. 3D volume rendering was performed using MARS-Vision software [1]. Artefact reduction was quantified in the MARS multi-energy images using contrast to noise ratio (CNR) and percentage of cupping effect.

RESULTS AND DISCUSSION
Reduction in streak artefacts and cupping effect were achieved through the narrow high energy acquisition using MARS. This complies with the physics of x-ray transmission where high energy x-rays can penetrate dense objects like metal implants. CNR improved in energy bins with lesser artefacts. A reduction of cupping effect from 23% to 1% in Al phantom and 32% to 6% in Ti phantom was noticed through the narrow bin MARS acquisition.

CONCLUSIONS
MARS imaging modality has the potential to provide better visualization of bone/tissue surrounding metallic implants. Existing modalities like dual-energy CT rely on extrapolated high energy images to reduce metal artefacts. Photon counting technology offers direct ways to measure high energy information through energy binning. Future work includes obtaining direct material information using specialized reconstruction methods which can virtually eliminate artefacts related to beam hardening.

REFERENCES
DAY 2

SESSION 1 – ECR AWARD
Quantification of the Vastus Lateralis Obliqus Muscle Size Using MRI: Addressing Inconsistency in the Definition of the Quadriceps Muscles

Geoffrey Handsfield, Katherine Knaus, Joseph Hart, and Silvia Blemker

INTRODUCTION
Within the quadriceps muscle group, the posterior and distal aspect of the vastus lateralis (VL) muscle is structurally distinct from the proximal anterior portion of the VL and applies supero-lateral forces on the patella. Its structural distinctness has led to its historical definition as a second head of the vastus lateralis muscle, the vastus lateralis obliqus (VLO) [1]. Definitions of the quadriceps muscle group are often inconsistent, however, with some definitions ignoring the VLO altogether and others classifying the VLO as part of the vastus intermedius (VI) [2]. To date, the size of the VLO has not been examined in healthy subjects in vivo, leaving a gap in knowledge as to what are normal lateral forces on the patella. With increasing focus on patellar mal-tracking as a source of knee pain, there is a need to better understand VLO size and structure to appreciate how lateral muscle forces may contribute to knee dysfunction. In the present study, we use MRI to quantify VLO volume in 10 healthy individuals. Knowledge of this region of muscle may have implications in better classifying muscle architecture and its role in causing patellar mal-tracking and knee pain.

METHODS
Imaging data from ten healthy, recreationally active adults and adolescents (4 female, 6 male, height: 1.71±.07m, mass: 63.9±9.3kg, age: 21±7 years) from a previous study [3] were included in the present study. Original scanning was conducted on a 3T Siemens Trio scanner using a spiral gradient echo MRI pulse sequence [3]. Imaging data was collected from above the iliac crest to below the ankle joint in stacks of 5mm slices with 1.1×1.1mm in plane resolution. All protocols were approved by our institution’s Institutional Review Board. The quadriceps muscles were segmented by trained observers. The VL was subdivided into two regions: (1) the vastus lateralis longus (VLL) was defined as the region superficial to the vastus lateralis aponeurosis and (2) the vastus lateralis obliqus (VLO) was defined as the region deep to the vastus lateralis aponeurosis but distinct from the vastus intermedius (VI) muscle. In pilot cadaver dissection, VLO fibers originated on the VL aponeurosis and inserted into the superior and lateral aspect of the quadriceps tendon.

RESULTS AND DISCUSSION
All ten subjects imaged displayed a VLO muscle head as evidenced by muscle tissue deep to the VL aponeurosis and distinct from VI (Fig. 1). The VLO represented 21.3% of the vastus lateralis complex (5.7% standard deviation) on average and represented 11.3% of the entire quadriceps muscle group (3% standard deviation). The VLL, VM, and VI represent 41.8%, 28%, and 19% of the quadriceps respectively. Additionally, the VLO volume is 62% of the volume of the VI.

CONCLUSIONS
The VLO is a reasonably large and identifiable head in the vastus lateralis complex. Its volume represents over 10% of the quadriceps group, indicating the potential for large muscle forces due to the VLO, which applies a lateral force on the patella. Mis-classification of VLO as VI will result in a 20% under-estimation of VL size and more than 60% overestimation of VI size, which may affect results from muscle models that rely on accurate estimates of muscle sizes.

ACKNOWLEDGEMENTS
We thank the UVA-Coulter Research Partnership for funding.

REFERENCES
Rapid Lower Limb Geometry and Muscle Insertion Estimation from Motion-Capture Landmarks

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INTRODUCTION

Rapid generation of lower limb bone models is essential for clinically applicable patient-specific gait modeling [1]. Simulations not only need bone geometry and pose, but also their muscle attachment sites. Accurate bone geometry and attachment sites are usually labouriously segmented from medical images, which are expensive and inconvenient to obtain. Motion-capture is a routine part of gait assessment but contains relatively sparse geometry information. We present a workflow, implemented in an easy-to-use software application, which uses a statistical model to accurately estimate lower limb bone geometry, pose, and muscle attachment sites from seven commonly used motion-capture landmarks. Our method can significantly reduce modelling time and increase the feasibility of clinical gait modelling.

METHODS

An articulated statistical shape model of lower limb bones (pelvis, femur, patella, tibia, fibula) was trained from 26 sets of segmentations of post-mortem CT images. Each bone was modelled as a parametric mesh (the atlas mesh) deformed to the geometry of each individual [2]. Principal component analysis on the 26 sets of meshes produced a statistical model of bone shape variation. Joint articulation was defined according to ISB standards [3] about standard anatomical landmarks embedded in the parametric space of each atlas mesh.

Also embed were muscle attachment regions obtained from SOMSO (Sonneberg, Germany) models of each bone. For each bone, the 3-D geometry of its Somso model was digitised using a laser scanner while attachment site boundaries were digitised using a FARO (Lake Mary, USA) digitiser. The bone's atlas mesh was fitted to the SOMSO geometry so that digitized attachment regions could be projected onto the mesh and embedded in parametric space.

Patient-specific meshes are produced by deforming the articulated lower limb atlas meshes to minimise the least-squares distance between the embedded landmarks and the motion-capture landmarks (LASIS, RASIS, sacral, medial and lateral femoral epicondyles, medial and lateral malleoloi). The deformation is a combination of rigid-body transformations of the pelvis and about each joint and a non-rigid transformation defined by the statistical shape model. Since attachments sites are embedded in the meshes, their anatomic positions are preserved during the deformation. This model generation method has been implemented as a plug-in for the open-source MAP Client software [4].

RESULTS AND DISCUSSION

Model generation accuracy was validated using a leave-one-out experiment where in turn, each of the 26 models was left out of the statistical shape model and used to provide the target landmarks and the ground-truth geometry. Mean landmark error was 3.28 mm. Mean surface error was 4.60 mm (pelvis), 4.61 mm (femur), 3.54 mm (tibia/fibula), and 4.58 mm (patella). Figure 1 shows good overlap of estimated and actual bone geometry for three randomly chosen individuals. The highest errors occurred around the greater trochanter due to anteversion variations.

![Figure 1: Examples of estimated bones (green) superimposed on ground-truth bones (yellow).](image)

CONCLUSIONS

We have developed a method for quickly and automatically generating patient-specific models of lower limb bones with muscle attachment sites. As a part of a MAP Client model generation workflow, the models can be output as geometries for gait simulations and other biomechanical models.

ACKNOWLEDGEMENTS

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REFERENCES

YIELD TORQUE IS COINCIDENT WITH CLINICAL TIGHTENING TORQUE.

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INTRODUCTION

Whether used alone or in combination with plates, bone screws are the most common implantable device. Clinically, screws are manually tightened to what they subjectively feel to be the ‘optimal’ torque, depending on the quality of the host material [1]. A surgeon’s ability to accurately gauge the appropriate level of tightening torque depends heavily on experience [2, 3], since there is no quantification as to what this torque should be. Previous studies have established that the average clinical tightening torque lies within the range of 84 – 88% T\text{max} [4, 5]. It has been suggested that the tactile feedback signaling for the surgeon to stop tightening is likely attributable to the onset of tissue yielding; however to date this has not been investigated.

METHODS

This study considered data sets that had been used in two previous studies [6, 7]. The first study considered a 6.5mm outer diameter (OD) cancellous lag screw with a 16mm thread length (Mathys, Australia). The screw was inserted into samples of either synthetic (n=142), ovine vertebral (n=68), or human femoral head bone (n=80). The latter study considered a 7.0 mm custom manufactured Aluminium screw inserted into excised human femoral head specimens (n=21). For all tests, the insertion torque, compression under the screw head and angular rotation were simultaneously recorded whilst the screw was inserted to failure.

Post analysis was performed using a custom written program (Matlab, Ma, USA). Head contact (HC) was defined once the slope of the compression trace exceeded a 10N threshold. Head contact torque (T\text{HC}) was defined as the average torque over the 60 degrees of rotation prior to head contact. Stripping torque was defined as the maximum torque (T\text{max}) measured by the torque transducer; yield torque (T\text{yield}) was determined using a 0.2 degree offset method as follows: the ‘linear’ region of the torque vs angular rotation was defined as the region of the curve between the tenth and fiftieth percentiles of T\text{HC} to T\text{max}. A line was constructed parallel to the slope of the linear region, but offset by 0.2 degrees. T\text{yield} was defined as the torque at which the constructed line intersected the smoothed torque versus rotation curve. Rotation angle between HC and yield and HC and T\text{max} were also calculated.

RESULTS AND DISCUSSION

Median, interquartile ranges (IQR) and coefficient of variation were calculated for each of T\text{yield}, T\text{max}, T\text{yield}/T\text{max} and rotation angle are listed in Table 1. The average T\text{yield}/T\text{max} for the human bone specimens appears coincident with the reported levels of clinical tightening torque with similar COV [4, 5]. T\text{yield}/T\text{max} was lower for both ovine and synthetic bone samples. The COV was largest for rotation angle suggesting the use of rotation angle may not be appropriate for determining the end point of screw insertion in cancellous bone.

Table 1: Results for analysis of torque versus rotation curves

<table>
<thead>
<tr>
<th></th>
<th>Human / SS screw</th>
<th>Human / Al screw</th>
<th>Ovine SS screw</th>
<th>Synthetic SS screw</th>
</tr>
</thead>
<tbody>
<tr>
<td>T\text{yield} (Nm)</td>
<td>(median, IQR) COV</td>
<td>(median, IQR) COV</td>
<td>(median, IQR) COV</td>
<td>(median, IQR) COV</td>
</tr>
<tr>
<td></td>
<td>1.50 (0.97) 0.50</td>
<td>1.85 (1.17) 0.42</td>
<td>1.10 (0.55) 0.43</td>
<td>1.37 (1.18) 0.61</td>
</tr>
<tr>
<td>T\text{max} (Nm)</td>
<td>1.84 (1.12) 0.45</td>
<td>2.04 (1.18) 0.38</td>
<td>1.54 (0.77) 0.35</td>
<td>1.76 (1.53) 0.54</td>
</tr>
<tr>
<td>T\text{yield}/T\text{max}</td>
<td>0.85 (0.16) 0.12</td>
<td>0.89 (0.13) 0.11</td>
<td>0.71 (0.07) 0.17</td>
<td>0.78 (0.15) 0.13</td>
</tr>
<tr>
<td>Rot\text{HC-Tyield} (º)</td>
<td>38.9 (28.4) 0.54</td>
<td>38.8 (31.8) 0.52</td>
<td>56.9 (58.3) 0.74</td>
<td>27.4 (16.9) 0.42</td>
</tr>
</tbody>
</table>

CONCLUSIONS

This study demonstrate that the median ratio of T\text{yield}/T\text{max} is consistent with the clinical ratios of tightening torque/T\text{max} reported in the literature and that a set percentage of T\text{max} may be a more reliable end point than rotation angle.

ACKNOWLEDGEMENTS

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REFERENCES

Quantification of articular cartilage health using MARS spectral computed tomography
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INTRODUCTION
Non-invasive imaging has become increasingly important in detection of cartilage damage, osteoarthritis (OA) progression, as well as for determining the quality of cartilage repair strategies. Current imaging methods are semi-quantitative or have limitations in detecting early osteoarthritic changes and cartilage lesions [1]. A sensitive, non-invasive method for quantifying biomarkers of cartilage health and tissue quality would have significant clinical impact as a diagnostic tool. Glycosaminoglycans (GAGs) are an important constituent of cartilage extracellular matrix. GAG content is also reduced in OA making it an important biomarker for cartilage health.

The Medipix All Resolution System (MARS) is a novel spectral computed tomography (CT) imaging technology [2] that uses a photon counting detector to discriminate and quantify multiple materials simultaneously in a single imaging modality. The aim of this study was to validate the ability of MARS spectral-CT to allow quantitative 3D imaging of GAG content in healthy and osteoarthritic tissues.

METHODS
Quantitative 3D analysis of GAG content was investigated using a negatively charged ionic iodine contrast agent (Hexabrix). Healthy bovine cartilage-bone explants (8mm) as well as excised human tibial plateaus (8mm coronal plane sections) from OA patients undergoing total knee arthroplasty were incubated in Hexabrix. MARS spectral scans were performed, and 3D images were reconstructed. Material decomposition was applied to separate the materials within the sample, and the amount and distribution of contrast agent was quantified. Histological staining (Safranin-O) and destructive biochemical assays for GAG quantification (DMMB dye) were performed for direct comparison with spectral data.

RESULTS AND DISCUSSION
We successfully demonstrated that spectral imaging combined with an ionic contrast agent (Hexabrix) could identify the distribution of negatively charged GAGs through the thickness of healthy cartilage as well as the distribution across a tibial plateau affected by OA (Fig 1). Analysis of healthy bovine cartilage-bone explants demonstrated a direct inverse correlation between GAG content and iodine concentration in cartilage as measured via destructive biochemical assay techniques and spectral analysis respectively.

Figure 1: Lateral human tibial plateau. A: macro photograph pre-scanning, B: attenuation via conventional CT, C: material decomposed images (magenta = Hexabrix, increasing colour intensity indicates decreasing GAG concentration; White = calcium in bone).

The zonal distribution of iodine contrast agent in reconstructed MARS images of healthy and OA cartilage was also consistent with GAG distribution observed in histological sections. Furthermore, segmentation of high contrast iodine in cartilage and high contrast calcium in subchondral bone was visualized using the MARS scanner (Fig 1C).

CONCLUSIONS
In this study, we demonstrate that key biomarkers of cartilage health can be quantitatively measured in both osteoarthritic and normal tissues using spectral CT. We demonstrate a method for non-destructively imaging cartilage and bone while obtaining quantitative information on GAG distribution within the tissue. This technique could potentially be used for nondestructive early assessment of osteoarthritic changes, or for assessment of cartilage repair tissue.

ACKNOWLEDGEMENTS
This work was supported with funding from Arthritis NZ and the Ministry of Business, Innovation and Employment.

REFERENCES
KEYNOTE 3 – Professor Hala Zreiqat
Development of unique ceramic scaffolds and nanoparticles with versatile modular platform for growth factor and drug delivery into bony environments

Hala Zreiqat
Faculty of Engineering and Information Technology, University of Sydney

An ongoing challenge in bone tissue engineering is to treat large bone defects under load. A wide variety of 3D scaffolds of different structures and material properties has been reported in the literature for bone regeneration; however, these have struggled to meet the requirements for adequate pore geometry and bioactivity combined with the mechanical strength necessary for bone regeneration under load. Bone is able to achieve these properties via its unique anisotropic structure and truss architecture. We have taken a step towards meeting the combined requirements for bone regeneration under load through our development of the Sr-HT Gahnite ceramic, which is bioactive. We used a three dimensional (3D) printing technology to fabricate glass-ceramic scaffolds with distinct pore geometries, which simultaneously display the properties of high mechanical strength and bone-like architecture. A particularly promising combination was Sr-HT Gahnite scaffolds with a hexagonal pore structure, which provided compressive strength of 110 MPa (comparable to cortical bone) at high porosity (70%) and interconnectivity (100%). Enhanced load transfer, high fatigue resistance and improved flexural strength were also noted. Importantly, at similar porosity, the compressive strength recorded for our Sr-HT Gahnite scaffold with hexagonal geometry was higher than the values reported for polymeric and composite scaffolds by 150-fold, as well as ceramic and bioactive glass scaffolds (in clinical use or under development) by 5-fold. Such scaffolds with optimised pore geometry opens avenues for treatment of load bearing bone defects in various clinical applications including orthopaedics, dental and maxillofacial.

Nanoparticle-based drug delivery systems (DDs) are a rapidly growing field of interest for effective targeted drug delivery application. We have given emphasis to calcium phosphate (CaP) based nanoparticles (NPs) due to their excellent biocompatibility and biodegradability properties. Here we report on the development of unique CaPs NPs with a range of micro-meso and macro porosities that offer a versatile modular platform for drug, gene and protein delivery. These could potentially be used for bone regeneration when biofunctionalised to induce local bone formation.
SESSION 2 – PhD AWARD
SORTING NEXIN 27 LINKS PTHR TRAFFICKING TO THE RETROMER FOR POSTNATAL BONE GROWTH

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INTRODUCTION
During postnatal life, longitudinal bone growth relies upon the ability of its cellular residents to receive and respond to local and systemic stimuli through a complement of transmembrane signaling receptors (cargo). The parathyroid hormone receptor type 1 (PTHR) is a class B GPCR critical for bone growth, remodeling and mineral ion metabolism. In response to stimulation, PTHR is rapidly internalised and recycled from early endosomes to the plasma membrane (PM) via the retromer sorting ensemble.

Sorting Nexin 27 (SNX27), a PDZ-domain containing member of the Phox-homology (PX) family of SNXs, is an endosome-associated cargo adaptor that operates in unison with the retromer to ensure the fidelity of cargo selection during activity-dependent endosome-to-PM retrieval.

METHODS
Through the combination of co-immunoprecipitation, isothermal titration calorimetry (ITC) and confocal microscopy, we identified a direct interaction between SNX27 and PTHR. Furthermore, through micro-CT and histomorphometry, we were able to examine the skeletal defects in SNX27 knock-out mice.

RESULTS AND DISCUSSION
By co-immunoprecipitation and ITC, we demonstrate that SNX27 and PTHR form a direct interaction whose affinity is enhanced by an order of magnitude in the presence of the retromer. At the atomic level, we demonstrate that PTHR associates electrostatically with the binding pocket of SNX27-PDZ domain via its canonical C-terminal PDZ-binding motif (PDZbm: E-T-V-M) and identify several upstream amino acids critical to the high affinity SNX27-PDZ: PTHR-PDZbm interaction. In response to agonist stimulation, we demonstrate that PTHR is internalised into early endosomes, an event that coincides with the rapid mobilization of SNX27 and recruitment of the retromer complex. Through depletion of SNX27-retromer via shRNA knockdown, we further show that PTHR is misrouted to lysosomes causing a net reduction of PTHR expression at the cell surface. Through micro-CT and histomorphometric analyses of 4-week-old SNX27 knock-out mice, we demonstrate that mice lacking SNX27 exhibit severe disturbances in postnatal bone growth, with a drastic reduction in bone size and net volume, decreased trabecular number and a conspicuous growth plate defect. Mechanistically, we demonstrate that these abnormalities are attributable, in part, to cell-autonomous disturbances in the signaling, trafficking and function of PTHR.

CONCLUSIONS
Therefore, SNX27 serves as an endosomal cargo adaptor that links PTHR trafficking to the retromer to preserve bone growth and homeostasis throughout postnatal life.

ACKNOWLEDGEMENTS
This work was supported from grants from the National Health and Medical Research Council of Australia (NHMRC), the Department of Health Western Australia, NHMRC Career Development Fellowship and by the Australian Research Council Future Fellowship. All microscopy was carried out at the Centre for Microscopy, Characterisation and Analysis, UWA. We also acknowledge the support of the resources and staff of the University of Queensland Remote Operation Crystallisation and the Australian Synchrotron with X-Ray diffraction data collection.
REGIONAL VARIATIONS IN PROXIMAL TIBIA BONE MICROARCHITECTURE AND JOINT LOADS IN END-STAGE KNEE OSTEOARTHRITIS

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2School of Health Sciences, University of South Australia, Adelaide, SA 3Department of Orthopaedics and Trauma, Royal Adelaide Hospital and Discipline of Orthopaedics and Trauma, University of Adelaide, SA 4Department of Orthopaedic Surgery, Repatriation General Hospital, Adelaide, SA, Australia

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INTRODUCTION

In human knee osteoarthritis (OA), local changes in bone mineral density in the tibia as measured by dual X-ray absorptiometry are suggested to be related to abnormal in vivo joint loading [1]. The relationship between in vivo measures of knee joint loading and changes in subchondral bone 3D microarchitecture, however, has not yet been explored. The subchondral bone, a shock absorber, protects the overlying cartilage against damage due to excessive loading.

The aim of this ongoing study is to examine, on end-stage OA patients undergoing total knee replacement (TKR), the relationships between knee joint loads measured in vivo using gait analysis prior to surgery, and variations in bone microarchitecture of the excised knees quantified with 3D micro-computed tomography (micro-CT).

METHODS

Patients: Fourteen knee-OA patients scheduled for TKR surgery (age 65±7 years) underwent pre-operative gait analysis.

Gait Analysis: The following kinetic variables were collected with 12 Vicon cameras and 4 force platforms and analysed with Visual3D v5 (C-motion Inc., MD, USA): knee external (ERM) and internal rotation moments (IRM), knee adduction moment (KAM) and knee adduction moment impulse, all normalized by bodyweight. The peak tibio-femoral joint contact force was calculated with a musculoskeletal model [2].

Micro-CT Analysis: After surgery the entire tibial plateaus were retrieved and scanned with micro-CT (17 µm / pixel, Skyscan 1076, Skyscan-Bruker, Belgium). The following subchondral bone 3D microarchitectural parameters were analysed in 4 cylindrical subregions of interest (ROIs, 10 mm diameter, 4 mm length) selected via software (CT Analyser, Skyscan-Bruker) in the antero-medial (AM), antero-lateral (AL), postero-medial (PM) and postero-lateral (PL) condyle: bone volume fraction (BV/TV), trabecular thickness (Tb.Th), number (Tb.N), separation (Tb.Sp), and structure model index (SMI).

Statistics: Subregional differences in microarchitectural parameters were tested by Kruskal Wallis followed by Mann-Whitney U tests. Associations between measurements from gait analysis and bone microarchitecture were investigated using Pearson’s correlations.

RESULTS AND DISCUSSION

Statistically significant differences (p < 0.05) in subchondral bone microarchitecture were found among ROIs. The AM ROI exhibited highest BV/TV (up to +83%), Tb.N (up to +48%), and lowest SMI (up to -74%), Tb.Sp (up to -25%) compared to AL, PM and PL ROIs. Tb.Th was greater (up to +25%) in AM than in AL and PL ROIs. BV/TV in AM and PM ROIs was negatively correlated with the peak ERM (r= -0.80, p < 0.01, Fig. 1b, and r= -0.69, p < 0.01). Positive trends were observed for ‘BV/TV vs. peak KAM’ (r =0.35, p = 0.23) and ‘BV/TV AM vs. KAM impulse’ (r=0.45, p = 0.11), and a negative trend for ‘BV/TV vs. peak IRM’ (r= -0.55, p = 0.054).

CONCLUSIONS

Our results suggest that, during stance, the peak ERM is negatively associated with BV/TV in the AM and PM tibial plateau. These associations with bone volume fraction found in the medial tibial regions could be linked to an adaptation of the bone due to altered loading patterns that generate increased stresses in this condyle [3]. Further analysis is required to elucidate these relationships.

ACKNOWLEDGEMENTS

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REFERENCES

INTRODUCTION
Long term repair of damaged articular cartilage represents a major challenge [1]. The combination of high-throughput cell-aggregate or micro-tissue fabrication methods with 3D printed scaffolds as bottom-up approaches for tissue engineering of cartilage constructs are emerging strategies. In addition to opening possibilities for high throughput screening, these strategies also promote high numbers of cell-cell interactions as well as cell differentiation capacity. However, fabrication of large, complex 3D constructs with precise arrangement of micro-tissue and scaffold components has been limited. Furthermore, no technology has yet been developed to automate the fabrication and delivery of 3D micro-tissues with 3D Printing [2]. We aimed to develop an automated 3D micro-tissue assembly system for fabricating tissue-engineered constructs efficiently without adversely affecting cell viability.

METHODS
An automated 3D micro-tissue assembly system consisting of a fluidic based singularisation and injection module was designed and incorporated into a commercial 3D Bioprinter (SYS-ENG, Germany). The singularisation module is able to process multiple micro-tissues and deliver them one-at-a-time to an injection module (via custom LabView control software), which then inserts the micro-tissue to a specific location in a 3D printed scaffold. Human nasal chondrocytes were isolated, expanded to passage 3, and Ø1mm micro-tissues were formed using a high throughput 96-well plate format in chondrogenic differentiation media as described previously [3]. The efficiency of the device was determined by measuring the number of micro-tissues that could be successfully singularized and delivered to the injection module (n=100). Bright-field microscopy was used to measure any physical deformation of micro-tissues during the automated process, and live/dead and trypan blue exclusion assays were used to quantify cell viability (n=4).

RESULTS AND DISCUSSION
Singularisation efficiency was determined to be 97%±6.6 demonstrating the fluidic-based system for singularisation and delivery of individual micro-tissues was highly efficient. Comparison of bright-field microscope images of micro-tissues revealed that there was no significant difference in size and shape (p>0.05) before and after singularisation. Live/dead (Fig. 1a) and trypan blue exclusion (Fig. 1b) assay revealed no significant difference in viability between control and micro-tissues handled using the device. Finally, a 3D printed PEGT/PBT polymer scaffold (3.8x3.8x3.2mm; 1mm fiber spacing) was fabricated as described previously [1, 3] and a tissue construct containing 9 micro-tissues was successfully assembled using the system (Fig. 1c).

CONCLUSIONS
We demonstrated a novel and efficient system for the automated assembly of micro-tissues in 3D printed scaffolds without deforming or significantly affecting the viability of the micro-tissues. This technology paves a pathway for biofabrication of large assembled tissues with complex 3D architecture and of clinically relevant size and shape.

ACKNOWLEDGEMENTS
The authors wish to acknowledge Julian Murphy and Manfred Ingerfeld from University of Canterbury and funding from the Royal Society of NZ Rutherford Discovery Fellowship (TW).

REFERENCES
INTRODUCTION

Inappropriate mechanical loading of the Achilles tendon causes tendinopathy leading to locomotor impairment. Locomotion requires the coordinated activation and control of multiple muscles by the central nervous system (CNS). Evidence suggests that the CNS uses a modular organisation to activate muscles, i.e. muscle synergies, to execute different locomotor tasks in humans and animals [1]. Muscle synergies are sets of excitation primitives (XPs) and muscle weightings of lower dimensionality with respect to the number of muscles [2]. Although rarely studied, common synergies have been observed across different motor tasks like walking, running, and swimming in humans, cats and frogs [1]. It is unknown, if these synergies are specific or shared across different locomotor tasks in response to a musculoskeletal injury like Achilles tendinopathy. Thus, we investigated if shared synergies existed across healthy and tendinopathy rabbits. To this end we reconstructed the muscle excitations of the tendinopathy rabbits from the muscle synergies of healthy rabbits. We also examined if generic Gaussian synergies, created from the combination of all locomotor tasks and rabbits, predicts the tendinopathy rabbits’ muscle excitations.

METHODS

Three healthy and four tendinopathy rabbits performed hop and box-jump locomotor tasks. Electromyograms (EMGs) were recorded at 1000 Hz from 5-to-7 bipolar fine wire electrodes inserted in each rabbit’s left hindlimb muscles, that resulted in EMGs from 8 muscles in healthy rabbits and 5 muscles in tendinopathy rabbits (Table 1). EMG data were band-pass filtered (30-400 Hz), full-wave rectified, and low-pass filtered (10 Hz) to create EMG linear envelopes, which were amplitude-normalised to the peak value in each trial and time-normalised to 100 points. Processed-normalised EMGs across all rabbits and trials were averaged and concatenated to create five EMG datasets: 1) healthy hop, 2) healthy box-jump, 3) tendinopathy hop, 4) tendinopathy box-jump, and 5) generic combined rabbit-task-pathology. Synergies were extracted using non-negative matrix factorisation [3] from each EMG dataset, with the number of synergy modules increased until the variation accounted for each individual muscle was greater than 80%. The XPs of the generic set were fitted with Gaussian curves. The synergies were then linearly combined using optimised weightings to best reconstruct the muscle-specific EMG patterns. The reconstructed EMGs were compared to the tendinopathy rabbits’ hop and box-jump averaged EMGs using Pearson correlations (R).

RESULTS AND DISCUSSION

The XPs from the tendinopathy rabbits’ hop and box-jump EMG datasets were similar (Fig. 1). The task-specific synergies from the healthy rabbits and generic Gaussian synergies predicted the tendinopathy rabbits averaged EMG linear envelopes for both locomotor tasks, except for Biceps as it was not recorded in healthy rabbits (Table 1).

CONCLUSIONS

High correlations between the averaged muscle excitations and reconstructed EMGs, using either healthy and generic datasets, indicate that tendinopathy does not affect the modularity of muscle recruitment patterns. Thus generic synergies can be used to construct a larger set of muscle EMGs than experimentally available to drive neuromusculoskeletal models to estimate Achilles tendon forces and strain distribution in rabbits with tendinopathy.

REFERENCES


Table 1: Correlations between tendinopathy task–specific averaged EMGs and reconstructed EMGs from two synergies.

<table>
<thead>
<tr>
<th>Muscles</th>
<th>Tasks</th>
<th>Hop</th>
<th>Box-jump</th>
<th>Hop</th>
<th>Box-jump</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy dataset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biceps</td>
<td></td>
<td>0.6</td>
<td>0.85</td>
<td>0.63</td>
<td>0.71</td>
</tr>
<tr>
<td>Tibialis Anterior (TA)</td>
<td>0.99</td>
<td>0.87</td>
<td>0.94</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Lateral Gastrocnemius (LG)</td>
<td>0.96</td>
<td>0.89</td>
<td>0.95</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Vastus Lateralis (VL)</td>
<td>0.7</td>
<td>0.36</td>
<td>0.83</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Generic dataset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biceps</td>
<td></td>
<td>0.92</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibialis Anterior (TA)</td>
<td></td>
<td></td>
<td></td>
<td>0.63</td>
<td>0.71</td>
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<tr>
<td>Lateral Gastrocnemius (LG)</td>
<td></td>
<td></td>
<td></td>
<td>0.94</td>
<td>0.93</td>
</tr>
<tr>
<td>Superficial Digital Flexor Muscle (SDFM)</td>
<td></td>
<td></td>
<td></td>
<td>0.95</td>
<td>0.91</td>
</tr>
<tr>
<td>Vastus Lateralis (VL)</td>
<td></td>
<td></td>
<td></td>
<td>0.83</td>
<td>0.76</td>
</tr>
</tbody>
</table>
INTRODUCTION
Injectable bone cements (IBCs) are an ideal form of bone graft substitutes to repair bone defects, as it allows for minimally invasive procedures and fills complex-shaped defects. Currently available IBCs such as polymethylmethacrylate (PMMA) and calcium phosphate cements (CPCs) possess unfavourable degradation and/or handling properties. We have previously developed strontium-doped hardystonite (Sr-Ca₂ZnSi₂O₇, SrHT) scaffolds with excellent in vivo bioactivity [1]. This study aims to develop an injectable SrHT phosphate cement (SPC) to address the need for a bioactive and resorbable IBC with good handling and injection properties.

METHODS
SrHT powder was mixed with sodium phosphate (NaH₂PO₄) and borax (Na₂B₂O₃·10H₂O) powders with a weight ratio of 1:1:0.03. Deionized water was added to the powder mixture at 0.25mL/g, and mixed thoroughly for 1min. Injectable after initial mixing time was measured as percentage of cement extruded through a standard 1mL plastic syringe over original cement mass. Cement cohesion after being injected into distilled water was also observed. The chemical composition of set SPCs were characterized under x-ray diffraction (XRD). Compressive testing of cylindrical SPC samples (0.5mm/min) was done after being immersed in deionized water for 2h, 1d, 3d and 7d. SPC discs were immersed in simulated body fluid (SBF) at 37°C at 3d and 7d. SPC discs were immersed for 1d and 7d were examined under scanning electron microscopy for surface apatite formation.

RESULTS AND DISCUSSION
SPC pastes demonstrated cohesive cement extrusion when injected into distilled water for up to ~8 mins post-mix, indicating initial setting time, and remained >97% injectable for up to 20mins. It was not possible to extrude the paste after 25mins, indicating its final setting time (Fig. 1). XRD showed three crystalline phases in SPC: SrHT, willemite, and calcium zinc phosphate, and a broad background peak indicating an amorphous phase. Compressive strength of SPCs reached 7.4±0.8MPa, 11.7±1.8MPa, 16.0±0.9MPa and 8.8±2.3MPa (±SD) after being immersed in 37°C deionized water for 2h, 1d, 3d and 7d respectively. Compressive modulus reached 448±43MPa, 784±119MPa, 939±91MPa and 952±172MPa at respective time points. The compressive values lie within the range of human cancellous bone. The pH profile of SPC in 37°C SBF remained relatively neutral at 7.2–7.5 at the measured time points up to 14d, and SPC discs showed weight loss of 12.0±1.4% and 35.5±5.0% after being immersed in 37°C SBF at 7d and 14d respectively (Fig 1. h). Complete degradation of IBCs after ~6 weeks has been reported to be ideal [2]. SPC discs showed extensive surface apatite formation when immersed in 37°C SBF after 1d and 7d, indicating potential bioactivity [3] (Fig. 2).

REFERENCES

Table 1: Comparison between SPC and clinically used IBCs. Red/green boxes indicate undesirable/desirable properties.

<table>
<thead>
<tr>
<th>PMMA</th>
<th>Apatite-based CPC</th>
<th>Brushite-based CPC</th>
<th>SPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomer</td>
<td>Bioactive ions Ca, P</td>
<td>Bioactive ions Ca, P</td>
<td>Bioactive ions Ca, Si, P, Zn, Sr</td>
</tr>
<tr>
<td>Permanent</td>
<td>Almost non-resorbable, &gt;6months in vivo</td>
<td>Resorption approx. in line with new bone growth, but also shown to form non-resorbable apatite</td>
<td>~35% weight loss in SBF in 2 weeks, in line for ideal 6-week resorption period</td>
</tr>
<tr>
<td>Toxic monomers</td>
<td>Significant filter pressing</td>
<td>Significant filter pressing</td>
<td>No filter pressing observed</td>
</tr>
<tr>
<td>Setting time 8–10mins</td>
<td>Injectable for 3–10mins</td>
<td>Very short setting time (~3mins)</td>
<td>Injectable for 3–7.5min</td>
</tr>
<tr>
<td>High modulus (2–3 GPa)</td>
<td>High modulus (1–3GPa)</td>
<td>High modulus (1–3GPa)</td>
<td>Both compressive strength and modulus in range of surrounding cancellous bone</td>
</tr>
</tbody>
</table>
DAY 3

PODIUM 4
A NEWFINITE-ELEMENT SOFTWARE PIPELINE FOR THE MICRO-STRUCTURAL ANALYSIS OF THE PROXIMAL FEMUR
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INTRODUCTION
Understanding how the trabecular and cortical compartments contribute to the load-bearing capacity of the human proximal femur has important implications to both clinical practice and basic-science [1]. Advances in computing power and computed-tomography (CT) scanners now makes it possible to run large-scale micro-structural finite-element (FE) analysis of the femur, providing in-depth information on the trabecular and cortical bone mechanics. However, current microstructural femur models require large supercomputing power, ad-hoc software and the common point-load simplification of the hip contact force that lead to numerical artefacts in the femoral head region. We present a novel software pipeline for generating micro-structural FE models of the human femur, determine the hip pressure distribution on the femoral head, solve the elastic problem and post-process the large result file.

METHODS
An ex-vivo femur from a healthy woman (41 year old, 179 cm tall, 104 kg weight) was obtained from the Melbourne Femur Collection [2]. A stack of 1706 consecutive transaxial images, 1536 x 1536 pixels (82 µm isotropic pixel size) was obtained using a high-resolution pQCT (Xtreme CT, Scanco Medical, Bruettisellen, Switzerland). The new software pipeline included image processing (CT Analyser v1.14, Skyscan-Bruker, Kontich, Belgium), in-house pre- and post-processing and the preconditioned conjugate gradient (PCG) solver implemented in ANSYS (ANSYS Inc., USA). Pre-processing involved segmentation of bone voxels using uniform thresholding (min=96, max=255) and a 3D-sweep algorithm to remove isolated voxels (speckles) (software CT.Analyser). The linear hexahedrons mesh was generated using an in-house lossless variation of the Vertex Pooling algorithm [3]. The hip contact pressure distribution was calculated using an in-house routine by (a) defining a superficial element layer using Delaunay triangulation, (b) assuming Hertzian pressure distribution and (c) using the average hip contact force during single leg stance [4]. The model was fully constrained at the most distal element layer and solved by setting a convergence tolerance of 1e-6. The mesh generation speed was assessed by generating a 1.45 billion Degree-Of-Freedom (DOF) model using a stack of 500 images, 984x984 pixels, binarized as a full homogenous solid. The resultant of the hip contact pressure distribution was compared to the input point force. Simulations were run on a single PC with 512 GB memory (RAM) and 8 CPUs shared-memory parallel computation procedure. Time to solution was recorded and compared with published state-of-the-art micro-structural FE models of the femur [5]. The von Mises stress was visualized over a coronal cross-section using the ANSYS built-in tool as well as with an in-house Matlab routine.

RESULTS AND DISCUSSION
The femur model was a 333 M DOFs model, generated in 22 minutes. The 1.45 billion DOFs model was generated in 2.7 hours, requiring 4.7 GB memory. The resultant of the pressure distribution differed by less than 26.5 N from the input point force. The PCG solution required 94 CPU hours, 6 processors and 441 GB of memory. These results compare favourably with the largest published microstructural FE femur model [5], which required 722 CPU hours, 2176 processors and 10 TB of memory for solving an 840 M DOFs model, using an algebraic multi-grid system [5].

Figure 1: The von Mises stress during single leg stance.

Visualization of a femur cross-section (Fig. 1) of the element results required ~4 hours using the ANSYS built-in tool, compared to ~3 seconds using the in-house Matlab routine.

CONCLUSIONS
The new software pipeline is a valid solution for generating, solving and post-processing micro-structural models of the human femur.

ACKNOWLEDGEMENTS
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REFERENCES
BIOMECHANICAL EVALUATION OF A SELF-SUPPORTED ONE-HANDED LIFTING TECHNIQUE IN HEALTHY SUBJECTS

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INTRODUCTION
Low back pain (LBP) affects nearly 80% of Australians with estimated annual costs of $9.17 billion [1]. LBP can result in difficulty bending and lifting objects. Anecdotally, patients with LBP who lift low-to-medium mass loads with one hand while bracing their other hand against the corresponding thigh, report reduced pain during occupational and daily living activities (Fraser, personal communication). Lumbar spine loads resulting from bracing on various external supports have been estimated [2,3], but self-supported bracing against the thigh has not been assessed. Anecdotally, patients report reduced pain during occupational and daily living activities (Fraser, personal communication). The aims of this project were: to determine hand-thigh forces for a self-supported thigh bracing technique; and, to compare L5/S1 net joint loads, and lumbar rotation, for the Braced technique vs. unsupported One-handed, Two-handed, and Self-selected strategies.

METHODS
11 healthy participants (6M/5F, age 26.3±7.8, BMI 25.1±4.2), with no history of LBP, lifted a 1 kg mass using four strategies: self-selected; two-hand; one-hand; and, braced one-hand (Figure 1). A 12-camera motion capture system (MX-F20, Vicon), two force platforms (BP600400, AMTI) and custom apparatus incorporating a load cell (9327C, Kistler) strapped to the distal thigh, were used to record kinematics, ground reaction forces and bracing forces, respectively. Net joint loads at L5/S1 (signed RMS), and lumbar spine rotation angle (peak), were estimated using a 3D rigid body model (Visual3D, C-motion) and compared between strategies with repeated measures ANOVA (α≤0.05) and post-hoc paired t-tests.

RESULTS AND DISCUSSION
Peak bracing loads were 198±57 N, 82±39 N, and 27±16 N, in normal, distal and medial directions (relative to thigh), respectively. L5/S1 axial (compression) load was lower for the Braced than the Self-selected (21±22%, p=0.004), One-hand (19%±22%, p=0.006), and Two-hand (19%±22%, p=0.007) strategies (Figure 2). The Braced strategy produced lower L5/S1 lateral bending moment than the One-hand (58%±51%, p=0.031) and Two-hand (68±38%, p=0.002) tasks. Other net joint loads and peak lumbar rotation were not significantly different across strategies.

CONCLUSIONS
The self-supported Braced strategy reduced axial forces (compared to three other strategies) and lateral moments (compared to one- and two-handed strategies) in the lumbar spine. Variability in between participants may be due to variability in foot placement, bracing posture and participant-specific neuromuscular control. Future studies will include a low back pain population and evaluate lumbar curvature, muscle activation and pain indices.

ACKNOWLEDGEMENTS
This project was supported in part by the 2014 Augusta Zadow Scholarship, SafeWork SA. Claire Jones is supported by an NHMRC Early Career Research Fellowship.

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Figure 1: Photos depicting the four bending & lifting strategies tested, at the point of picking up the mass.

Figure 2: Normalised signed RMS forces in bend-lift cycle for self (S), two-(T) hand, one-(O) hand, braced (B) strategies.

1 kg: 5 lifts
1 kg: 5 lifts per task (randomised order)
A COMPUTATIONAL FRAMEWORK TO PREDICT TIBIOFEMORAL JOINT KINEMATICS
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INTRODUCTION
Estimating kinematics and load sharing of the knee is critical to understanding the mechanical aetiology of knee disorders such as osteoarthritis. The kinematics of the tibiofemoral joint are controlled by a complex combination of soft tissue constraints and articular contact. Joint kinematics also play a vital role in determining the lengths and moment arms of muscles crossing the knee, which, in turn, alter the predicted muscle and joint contact forces [1]. Finite element models derived from magnetic resonance imaging (MRI) offer a promising method to account for subject-specific geometry and incorporate the relevant soft tissue structures, such as menisci, ligaments, and muscle. However, selecting the appropriate material properties as well as origins and insertions of ligaments remains a challenge [2]. The purpose of this research was to develop a finite element (FE) model of the tibiofemoral joint using non-weight bearing MRI to predict 6 dof knee kinematics.

METHODS
Sagittal plane supine MRIs of the right knee from one healthy male subject were manually segmented using a custom Matlab script. The geometrical boundaries of the bony segments, the articular cartilage layers and the attachment points of their soft tissue connectors were digitized, including: anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), lateral collateral ligament (LCL), medial collateral ligament (MCL), popliteofibular ligament (PFL), oblique popliteal ligament (OPL) and medial and lateral capsule (PCAP). Cubic Hermite elements were fit to the segmented point clouds of the bone+cartilage layer and hexahedral 8 node-element meshes generated using CMISS (Figure 1A, University of Auckland). Ligament bundles were modelled as discrete non-linear spring elements in FEBio (Utah University). Rigid body contact was assumed and the initial material properties of the ligaments were taken from the literature [3]. A non-linear optimisation algorithm was then used to calibrate ligament stiffness and reference strains to match whole joint knee laxity profiles taken from the literature [3]. To do this we performed ‘virtual’ laxity FE simulations using FEBio for anterior-posterior tibial translation and internal-external tibial rotation at 0 and 90 deg of knee flexion [2]. Using ligament stiffness values from the calibration we were then able to predict knee kinematics from 0 to 90 deg flexion (10 deg increments) by constraining the femur and leaving the tibia unconstrained in 5 DOF (flexion prescribed) and performed a quasi-static analysis to ensure static equilibrium. Predicted kinematics were then compared with those from the literature [3].

RESULTS AND DISCUSSION
Model predictions of knee kinematics were within the envelope of passive knee joint motions obtained from the literature (Figure 1B) and matched the knee laxity data, similar to what had been shown previously [2]. Discrepancies between model predictions and experimental measurements were expected as our model was developed from a different dataset to the experimental measurements. However, the calibration enabled a close estimate of the joint laxity profile. We plan to further validate the model by comparing model predictions of kinematics with weight-bearing MRI’s taken from the same subject. We also plan to include a meniscus to the model, which will influence the predicted contact pressures.

CONCLUSIONS
We have presented a model that is capable of predicting subject-specific kinematics of the tibiofemoral joint using only imaging data from a static, non-weight bearing MR image. The model has potential to inform rigid body musculoskeletal models as well as estimate the load distribution and cartilage stress within the tibiofemoral joint.

ACKNOWLEDGEMENTS
We gratefully acknowledge the financial support of the Royal Society of NZ Marsden Fund (UOA-1211).

REFERENCES
INTRODUCTION
Standard bi-cortical approaches to midshaft clavicle fixation pose a considerable complication risk due to the proximity of screws with the subclavian vessels and the brachial plexus, including fatal air embolisms, vascular and nerve rupture [1, 2]. Utilising uni-cortical screws in clavicle plate fixation reduces the risk of damage to neurovascular structures; however, poor resistance to bending and torsional loading has limited its application in the clinical setting [3]. In the present study a novel uni-cortical approach to plate fixation is presented. This repair utilizes long, oblique unicortical screws at the distal aspect of the plate which pull the fracture segments together and provide increased screw purchase. By employing a multiplanar purchase, combining both locking and non-locking uni-cortical fixation, it was hypothesised that this new clavicle fixation approach would demonstrate equivalent mechanical strength compared to gold standard bicortical approaches under both torsion and bending loads.

METHODS
Thirty clavicles were harvested from human cadavers. Clavicles were fractured at their mid-length with a transverse-plane osteotomy, and randomly assigned to one of three surgical fixation techniques: (i) bi-cortical fixation with non-locking screws (ii) bi-cortical fixation with locking screws, and (iii) a hybrid uni-cortical locking fixation, with terminal oblique non-locking screws. All repaired clavicle specimens were first tested non-destructively in torsion, then destructively in cantilever bending. Both sternal and acromial ends of the clavicle were potted in custom-built fixtures using dental plaster, and attached to an Instron Materials Testing Machine (MTS) using custom-designed fixtures. With the sternal end of the clavicle rigidly fixed, the acromial end of the clavicle was twisted by applying angular motion at a constant rate of 0.5 degrees/s until a torque of 9 Nm was reached (60% of the fracture torque) [4] (Fig 1A). Specimens were then reconfigured on the MTS for cantilever bending with the clavicle supported medially (Fig 1B). Bending was applied by displacing the free end of the clavicle down at a constant rate of 0.5 mm/s until a bending moment of 13.7 Nm was reached (60% of the fracture moment).

RESULTS AND DISCUSSION
The hybrid approach displayed a significantly lower mean torsional stiffness value when compared with locking bicortical plated constructs (mean difference: 134.4 Nm/degrees, confidence interval (CI), [32.3, 236.4], p=0.007). There were no significant differences between fixation constructs in either bending stiffness Nm/degrees (p=0.20) or ultimate bending moment Nm (p>0.79). The most common mode of failure in bending was fracture at the screw bone interface at the medial end of the clavicle.

CONCLUSION
A new uni-cortical hybrid approach to clavicle plate fixation produces similar biomechanical strength to conventional bi-cortical non-locking and bi-cortical locking techniques. By utilising screws that penetrate only one cortex, this procedure is a safe alternative to conventional bi-cortical clavicle plate fixation; however, excessive torsion may present failure risk. Future studies will focus on the long-term integrity and clinical outcomes of this repair.

REFERENCES
KEYNOTE 4 – Professor Gary Hooper
The ageing population will drive the direction of future health funding and as a result will have a significant influence on research funding in New Zealand and Australia. This group will increase the demand for innovative and new treatments to improve and maintain an active and functional lifestyle. Musculoskeletal conditions, such as arthritis, are more common in this age group and are likely to place an enormous socioeconomic burden on both countries. Within New Zealand the rate of hip and knee replacement is expected to increase 110% and 270% respectively by 2030. Politicians will be forced to address this increase and apportion more funds into both the treatment and research of these conditions. As a result research will become outcome driven with funding for treatment dependent on evidence based clinical results. Basic research will need to show that it is translational and able to improve these outcomes; however there remains a communication gap between basic research and the clinician which often presents a barrier to the successful clinical implementation of even the best innovations. The challenge is to successfully identify the barriers that contribute to this gap and bridge them.

This gap can be improved by addressing four main areas:
1. Identifying the actual clinical need.
2. Scientists and clinicians working together for improved outcomes
3. Maintaining a safe, ethical and moral landscape
4. Improving data bases to monitor outcomes

This presentation will address the above areas by concentrating on studies involved with treating arthritis; showing how scientist can work with clinicians, outlining the areas of need within our community, areas that clinicians perceive as ongoing problems, possible mechanisms for solving these problems and how we as scientists can improve the outcomes for patients.
PODIUM 5
INTRODUCTION
A common cause of orthopaedic implant failure is bone loss adjacent to the prosthesis. This is most frequently caused by prosthetic wear particles inducing an inflammatory response in the surrounding tissue which stimulates osteoclastic bone resorption.

A novel method for regulating this enhanced osteoclast activity is via the epigenetic modulation of gene expression through targeted suppression of specific histone deacetylases (HDACs). There are two main classes of HDAC enzymes with 11 isoforms that can have altered expression or activity in disease. We recently identified high HDAC 1 expression in large osteoclastic cells within human tissues obtained from sites of prosthetic loosening. These cells also contained prosthetic wear particles, most commonly polyethylene (PE) particles. This study aimed to assess the effects of a novel HDAC inhibitor (NW-21), designed to target HDAC 1, in human osteoclasts exposed to PE particles in vitro.

METHODS
Human peripheral blood mononuclear cells (PBMC) were isolated from 7 donor buffy coats obtained from the Australian Red Cross. The PBMC were subjected to a 24 hour rotating suspension of PE particles (2000µg/ml) in media to allow phagocytosis of the implant material. PBMC were then allowed to adhere to cell culture plates for 24 hours. The adherent cells (approximately 96% monocytes) were then cultured for 17 days. Receptor activator of nuclear factor kappa-B ligand (RANKL) was added to the media from day 7. Treatment of NW-21 (20nM in 0.01% DMSO) commenced from day 7.

Osteoclast formation and activity was analysed using tartrate resistant acid phosphatase (TRAP) staining. Osteoclast activity was assessed using a dentine resorption assay. Gene expression of osteoclast signalling, differentiation and activity factors, TNF receptor-associated factor6 (TRAF6), nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), calcitonin receptor (CTR), and B3-integrin were assessed on days 10, 14, and 17 using RT-qPCR. Expression of HDAC 1 and tumour necrosis factor-a (TNFα) were also assessed in this study.

RESULTS AND DISCUSSION
PE particles increased the number, size and resorptive area of individual osteoclasts. Inhibition of HDAC 1 with NW-21 significantly reduced the number of TRAP+ cells (p=0.0017) and inhibited resorption in the presence of PE particles.

Exposure to PE particles in vitro had no effect on the expression of genes involved in osteoclast signalling, TRAF6 or NFATc1. However, NFATc1 target genes related to osteoclast fusion and function, B3-integrin and CTR, were significantly upregulated by PE (p<0.05). HDAC 1 inhibition significantly suppressed these osteoclast factors (BE-integrin and CTR) in the presence of PE particles (p<0.05).

CONCLUSION and DISCUSSION
Pre-osteoclasts exposed to PE particles in vitro formed larger and more active osteoclasts, supporting the notion that prosthetic wear particles stimulate osteolysis and implant failure [1]. Interestingly, NW-21 suppressed PE particle induced formation and activity through factors involved in osteoclast fusion and function (B3-integrin and CTR). We observed no direct effect of NW-21 on the expression of genes related to osteoclast cell signalling, TRAF6 or NFATc1, as previously published in physiologically culture cells [2]. These findings show that HDAC 1 inhibition may suppress osteoclast related peri-implant bone loss.

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Early Osteoarthritis: mechanical and structural changes to the underlying bone and calcified cartilage

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INTRODUCTION
In the joint, bone is able to attenuate more of the joint loading force than the cartilage, due to its greater volume. The zone of calcified cartilage (ZCC) between the articular cartilage (AC) and the subchondral bone (SB) plays an important role in transmitting forces between these tissues with very different stiffnesses. The ZCC has been shown to be 100 times stiffer than the underlying articular cartilage and 10 times less stiff than the underlying subchondral bone.

Cartilage changes throughout osteoarthritis (OA) development are well characterised and in late stage OA the subchondral bone remodels, increases in bone fraction, and decreases in material density. However, reports of bone stiffness changes are conflicting. Furthermore, the calcified cartilage advances into the articular cartilage, forming duplicated tidemarks. Reports on the stiffness of the ZCC throughout OA are lacking, primarily due to the layer’s small volume.

This study considers the mechanobiological links between changes in the cartilage and bone by examining both mechanical and microstructural changes in the calcified cartilage and subchondral bone in early osteoarthritis.

METHODS
Using the bovine patella model of early OA [1], patellae displaying a range of degenerative states from an intact cartilage surface to moderate cartilage degeneration were studied. The mechanical properties of the ZCC and SB were tested using three scales of testing: macroscopic three point bending, microhardness indentation, and nanoindentation [2]. Structural changes in the AC, ZCC, and SB were examined using differential interference contrast (DIC) and scanning electron microscopy (SEM). Further, the proteoglycan content of the cartilage was qualitatively assessed using histology [1].

RESULTS AND DISCUSSION
Macroscopic mechanical testing demonstrated that the relative stiffness of the ZCC and SB changed with early degeneration. The bone was 10 times stiffer than the ZCC in healthy tissue, 5 times stiffer in tissues with mild degeneration of the articular cartilage, and 8 times stiffer in tissues with moderate degeneration. Further, microhardness testing demonstrated a gradual increase in stiffness across the osteochondral junction.

The microstructural analysis demonstrated the presence of structural irregularities along the osteochondral junction which contained vascular tissue and a cuff of bone (Figure 1). These are henceforth termed bony spicules [3]. The bone formation and directionality of these structures resemble developing osteons of bone remodelling.

CONCLUSIONS
The results suggest that biological responses occur in the underlying calcified tissues in the early stages of OA as indicated by alterations in microstructure and mechanical properties along the cartilage-bone interface. We propose that a mechanobiological link exists between these microstructural and material properties of cartilage, calcified cartilage, and bone which may play a role in the initiation and progression of osteoarthritis.

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REFERENCES
The mechanism of SHP in macrophage induced aseptic joint loosening by wear particles in vitro

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INTRODUCTION
Aseptic joint loosening is a key factor that reduces the life span of arthroplasty. There are currently few effective treatments for joint loosening except revision. We wanted to ensure whether SHP, a negative regulator of toll-like receptors (TLRs) signal pathway, plays an important role in aseptic joint loosening.

METHODS
Titanium particles in different sizes were prepared to stimulate macrophages. They were filtered into three groups according to diameter of particles, which average diameter were 0.82 ± 0.12 μ m(Ti-0.2), 3.75 ± 1.08 μ m(Ti-1.2) and 15.47 ± 5.18 μ m(Ti-10). After the particles stimulated the cells, Real-time PCR was utilized to demonstrate mRNA expression of target genes (TLR2, TLR4, SHP and TRAF6) and inflammatory cytokine in the murine macrophage cell line, RAW264.7, at different time-points (0.5h, 1h, 3h, 6h, 12h). We explored the inhibitory effects of SHP-targeted small interfering RNA (siRNA) on particle-induced inflammatory cytokine expression. siRNA targeting SHP were transfected prior to particle stimulation. Fluorescence microscopy showed that the efficiency of siRNA transfection were higher than 70%.

RESULTS AND DISCUSSION
Real-time PCR revealed mRNA expressions of TLR2, TLR4, TRAF6 and TNF-α in Ti-0.2 and Ti-1.2 group were peaking at 3h, 6h, 0.5h and 1h, respectively. In contrast, mRNA expression of SHP down-regulated at 0.5h and there was no significant difference in Ti-1.2 group at chosen time-points. TNF-α mRNA in the particle stimulation plus RNA interference (RNAi) groups were significantly higher compared with the particle stimulation-only groups (P < 0.05). Correlation analysis showed TNF-α in Ti-0.2 and Ti-1.2 group was positively correlated with TLR2 and TRAF6 while negatively correlated with SHP.

CONCLUSIONS
According to the studies above, mRNA of TLR2, TLR4, SHP, TRAF6 and TNF-α increased due to particles stimulation. Furthermore, smaller particles exhibited greater effect. However, SHP expression was depressed. TNF-α mRNA in RNA interference (RNAi) groups were significantly moved up. In conclusion, SHP was a negative regulator of macrophage induced aseptic joint loosening which involved TLR2, TLR4 and TRAF6.
INTRODUCTION
Microfracture technique and autologous matrix-induced chondrogenesis (AMIC) both invade the subchondral bone during treatment of cartilage defects. Manipulation of its architecture by microfracture could lead to deleterious effects and could impair cartilage healing. The aim of our study was to evaluate cartilage healing using both techniques and to report the histopathological changes in subchondral bone caused by microfracture technique.

METHODS
Eighteen skeletally mature female sheep were randomly assigned to 3 groups: AMIC, microfracture, and control. Eight millimeter diameter cartilage defects were created in the weight bearing area of the left medial femoral condyle. Treatment groups received 5 microfracture holes in the defects. Microfractured defects in the AMIC group were augmented with a collagen scaffold. Animals were euthanized at 26 weeks post-operatively and the stifle was processed routinely for histological evaluation. Cartilage healing was evaluated using a modified O’Driscoll scoring system (Maximum score: 27) and histopathological changes in the subchondral bone were evaluated.

RESULTS AND DISCUSSION
There was more repair tissue in AMIC (68% ±7%) and microfracture (63% ±25%) groups compared to the control (32% ±12%). The difference between treatment groups was not significant, but there was a significant difference between the control group and both treatment groups (p < 0.01). Median O’Driscoll scores were 12.5 and 14 for AMIC and microfracture, respectively. Histological assessment of the subchondral bone structure in treatment groups revealed presence of bone tunnels between the articular cartilage surface and the subchondral bone (fig. 1). Tunnels contained fibrinous exudate derived from the joint. However, no inflammatory cells were observed. Infill tissue of osteochondral defect regions was mainly composed of tissue from endochondral ossification, with cartilaginous tissue covering woven bone tissue matrix. In some animals, articular cartilage fragments, pushed down in the subchondral bone region by microfracture technique were detected (fig. 2). Fragments were surrounded by dense woven bone. Subchondral bone cyst formation was present in 11/12 (92%) animals that received microfracture techniques (fig. 3a). Cysts were often lined with dense fibrous tissue and blood vessels and hemorrhage were frequently observed. High numbers of osteoclasts and resorption lacunae were frequently present at the cyst periphery (fig. 3b). Cysts were often filled with fibrous tissue and some contained necrotic bone fragments. Bone surrounding cysts was sclerotic. Alterations induced by microfracture technique showed similarities with advanced osteoarthritis [1] and persistent communication between the joint cavity and subchondral suggests contribution of synovial pressure [2]. Cytokines within the synovial fluid could also contribute to osteoclastic bone resorption and cyst formation.

CONCLUSIONS
Although AMIC and microfracture techniques repair the articular cartilage surface by encouraging the ingrowth the cartilaginous tissue matrix via endochondral ossification, synovial fluid and cytokines penetrate into subchondral bone, which activates abnormal osteoclastic bone resorption. The latter may be the cause of cyst formation. Microfracture technique causes severe pathology of the subchondral bone which may be associated with the progression of osteoarthritis.

REFERENCES
CONFLICT OF INTEREST DECLARATION

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    The study was entirely funded by Orthocell. Minghao Zheng receives shares from Orthocell.
PROTEOMIC INVESTIGATION OF EARLY OSTEOARTHRITIC TISSUE DEGENERATION
Bincy Jacob, Viji Sarojini, Neil Broom, Mia Jullig and Ashvin Thambyah

INTRODUCTION
Early signs of osteoarthritis (OA) include subtle micro-to-nano scale structural changes in the joint tissues. These changes include de-structuring of the collagen fibril network, increasing mineralization at the cartilage-bone interface, and the advance of the bone cement-line [1-3]. Here we investigate the distinct proteomic profile associated with these structural changes using a validated bovine model of pre-OA [4]. We compare the protein profile of cartilage tissue showing signs of early structural degeneration versus intact cartilage tissue taken from the same joint.

METHODS
A total of fifteen bovine patellae were used in this study. These patellae were distributed into three groups: showing mild localized cartilage degeneration (n=7), moderate localized degeneration (n=5) and completely intact where there were no signs of degeneration (n=3). Importantly cartilage that had mild and moderate localized degeneration, despite some softening and swelling, still had relatively full thickness cartilage and was thus considered as early osteoarthritic [4]. Two cartilage blocks were sampled from each patella of all three groups. For those patellae showing localized degeneration, one block was obtained each from the degenerate location (lesion site) and from the neighboring intact-appearing tissue. Microstructural analysis was carried out on all samples using differential interference contrast optical microscopy (DIC) and histology was done to characterize tissue health using Mankin scoring. A comprehensive proteome analysis was performed via iTRAQ labeled liquid chromatography-tandem mass spectrometry.

RESULTS AND DISCUSSION
In the intact cartilage group, the paired intact samples showed only 3 proteins that were significantly different in their abundance levels- collagen IX alpha 3, tetranectin and osteoglycin. In patellae with localized degeneration, 166 proteins were identified and analyzed. Of this number, 46 proteins were found to be significantly different (p<0.05) between lesion and intact sites. Proteins that showed an increase in abundance, in all 12 degenerate samples compared to their 12 intact controls, included the following: Lumican, Apolipoprotein A-I, Thrombospondin-1, TGFβ1. Proteins that showed a decrease in abundance included: Lysozyme C, Glutathione S-transferase P and TIMP3. The full list of consistently ‘upregulated’ and ‘downregulated’ proteins, in all 12 degenerate samples compared to their 12 intact controls, is shown in Figure 1. The microstructure data showed that structurally-degenerate samples had a micro-level disruption in the superficial layer, increased fibrosity in the matrix (i.e. de-structuring), and bone spicule formation along the cement line.

This study uses a bovine model of cartilage tissue degeneration, and thus translation of the findings to human OA is limited. However this model of early degenerative tissue change in the bovine joint has been validated against human tissue [4] and thus allows for an initial study correlating between degenerative changes in tissue microstructure and protein levels.

CONCLUSION
Several key proteins known for their role in regulating fibrillogenesis, angiogenesis and new bone formation, have been identified in this study to be heavily involved in early osteoarthritic tissue degeneration.

ACKNOWLEDGEMENTS
The authors gratefully acknowledge the funding provided by Arthritis New Zealand.

REFERENCES
ORAL POSTERS 3
INTRODUCTION
Mechanical stimulation is known to be a determining factor in the maintenance and development of articular cartilage. Loading modalities such as dynamic compression and hydrostatic pressurisation have been seen to dictate the level of growth and organization of synthesized ECM within cell-seeded scaffolds for cartilage tissue engineering. Quantitative characterisation of the nature of this mechano-regulatory effect has great potential for the design of effective future tissue engineering strategies. Several studies have sought to model the role of biophysical stimuli on tissue regeneration for musculoskeletal tissues; most notably with regard to tissue differentiation during bone fracture healing [1, 2]. Multi-scale mechanical models use mechano-regulatory algorithms to simulate the adaptive response of tissue properties to local stress and strain profiles [3, 4]. It is thought that the techniques outlined here can be extended to facilitate the investigation of the role that mechanical stimulation plays in generating cartilage-like engineered tissue. Presented here is the methodology for the development of a predictive statistical model; the first stage in an overall multi-scale modelling framework.

METHODS
The experimental data used for this study concerns the fusion of human Articular Chondrocyte and Mesenchymal stem cell laden pellets within 3D scaffold architectures over a 21 day culture period. A semi-automated segmentation GUI was designed using MATLAS image processing toolbox to extract cell and Glycosaminoglycan (GAG) spatial distributions from histological images. Similarly, edge detection methods were used to obtain temporal changes in pellet shape from inversion scope images. This information was then sampled through inverse transformation methods that enable varied shape and spatial pattern generation. This informs a continuum mechanical mass diffusion model (ABAQUS finite element software) that simulates pellet fusion processes; pellet deformation and cell and GAG diffusion. A support vector machine learning approach trained on synthetic diffusion simulations permits the prediction of diffusivity from known experimental cell patterns. Mesoscale tissue growth response will be linked to a joint cartilage knee joint model to predict variations in homogenized cartilage tissue properties in response to joint loads.

RESULTS AND DISCUSSION
A probability distribution of cell arrangement was developed and used to predict cell variation (see Figure 1). This was repeated for cell pellet fusion pattern and GAG content. A morphology analysis of cell shape (using eigenvector analysis) also revealed different cell types and apoptotic cells (larger expanding cells). The morphological analysis allowed for better quantification of cell type and state than existing imaging classifications. Synthetic cell diffusion patterns were generated and the support vector machine learning was able to predict the most likely diffusivity for given experiment data.

Figure 1: Cell Pellet and Generated Cell distribution

CONCLUSIONS
This study presents the first stage of a multi-scale modeling pipeline to simulate tissue growth in cartilage tissue engineering scaffolds. A novel machine learning methodology trained on experimental histological data is used to ascertain how cartilage model parameters relate to tissue culture outcomes. It is thought that similar methods can be applied to experimental data concerning scaffolds under external mechanical loading. The inclusion of a biomechanical regulatory protocol within the outlined framework will permit further investigation of the complex role played by biophysical stimuli in the development of functional cartilaginous tissue.

REFERENCES
INTRODUCTION
Carpometacarpal (CMC) joint osteoarthritis (OA) is a serious and pervasive disease, affecting 15% of adults over 30 years and two to six times more women than men[1].

Sexual dimorphism, kinematics and their effects on joint biomechanics and resulting cartilage stresses are implicated with the pathogenesis of CMC OA [2]. Here we present an automated pipeline for creating finite element (FE) models of the CMC joint.

METHODS
A training set of 50 CMC joints were manually segmented from clinical CT scans of the hand with a resolution of 0.4x0.4x0.625 (age range: 18 yrs to 67 yrs; 24 females and 26 males). A custom piecewise parametric template mesh, designed with an articular surface division, was fitted to each segmented data cloud, resulting in a set of correspondent meshes of the metacarpal and trapezium bones. These were used to train a statistical shape model (SSM).

3D Haar-like features were sampled from the image about each mesh node and used to train a random forest regressor for each node (Figure 1 A). During segmentation, a mean mesh was fitted to nodal locations predicted by the regressors (Figure 1 B) using optimal deformations permitted by the SSM (Figure 1 C).

The articular surface division of the mesh was used to automatically build a uniform 3D hexahedral mesh of CMC joint cartilage. This cartilage mesh and the bone were finally imported into FEBio for FE analysis (Figure 1 D). FE analysis was performed using displacement driven static analysis. As a proof of concept, we simulated the stress through the cartilage in neutral position from clinical CT.

RESULTS AND DISCUSSION
It can be seen that the peak effective stress during static neutral position is 264kPa, with the median stress at 140kPa (Figure 2).

Figure 2: Effective stress in the CMC joint cartilage in neutral position

CONCLUSIONS
Our preliminary results are promising, indicating that this pipeline may be useful for the determination of stresses during different functional tasks.

ACKNOWLEDGEMENTS
This work was supported by the Nation Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health and the Auckland Bioengineering Institute.

REFERENCES
COMPARISON BETWEEN THE CANCELLOUS SCREWS PULLOUT STRENGTH AT THREE LEVELS OF TIGHTENING TORQUE IN THE HUMAN FEMORAL HEAD

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INTRODUCTION

Unintentional screw stripping may go undetected during fracture surgery, posing concern among surgeons of poor fixation. Clinically, surgeons estimate the level of tightening torque for optimal fixation based on their perception. However a surgeon’s perception of optimal tightening is not reliable for detecting and preventing stripping of the bone around the screw. Moreover, previous studies comparing the pullout strength (F\(_{\text{Pullout}}\)) of bone screws at different tightening torque levels showed contradicting findings on the optimal level of tightening torque for maximising F\(_{\text{Pullout}}\). Furthermore, these studies were performed only on cortical bones samples. No study has been performed in skeletal sites rich in trabecular bone, such as in the human femoral heads. The aim of this study was to compare the F\(_{\text{Pullout}}\) of cancellous screws in human femoral heads at three tightening torque levels after screw head contact.

METHODS

Following ethics approval and donor consent, 18 femoral heads (n=6 per group) of similar areal bone mineral density (mean±SD= 0.83±0.1g/cm\(^2\), p=0.06) and bone volume fraction (31±3%, p=0.54) obtained from hip replacement surgery (donor’s age= 77±11 years) were tested in this study. The specimens were subjected to screw tightening to an average±SD of 70±2 % of the predicted maximum (or stripping) torque (T\(_{\text{Max}}\)), 80±2 %T\(_{\text{Max}}\) and 90±3 %T\(_{\text{Max}}\) using a micro-mechanical test device capable of predicting T\(_{\text{Max}}\) from torque values gained during insertion. Pullout tests were then performed to measure F\(_{\text{Pullout}}\) using a servo- hydraulic materials testing machine. A one-way Anova analysis with Tukey HSD post-hoc was performed to compare the means of F\(_{\text{Pullout}}\) between the three groups. The significance level was set to p=0.05.

RESULTS AND DISCUSSION

F\(_{\text{Pullout}}\) for screws tightened to 80\%T\(_{\text{Max}}\) was significantly greater than for screws tightened to 90\%T\(_{\text{Max}}\) (F\(_{\text{Pullout}}\)=2.07±0.28 kN vs. 1.48±0.40 kN, p=0.019) and it tended to be greater than for screws tightened to 70\%T\(_{\text{Max}}\) (1.79±0.31 kN, p=0.33) (Fig. 1). F\(_{\text{Pullout}}\) at 70\%T\(_{\text{Max}}\) was greater than that at 90\%T\(_{\text{Max}}\), however not significantly so (p=0.27).

Figure 1: Boxplot of the F\(_{\text{Pullout}}\) of screws tightened to 70%, 80% and 90\%T\(_{\text{Max}}\). F\(_{\text{Pullout}}\) at 90\%T\(_{\text{Max}}\) was significantly lower to F\(_{\text{Pullout}}\) at 80\%T\(_{\text{Max}}\) (*p=0.019).

CONCLUSIONS

In this study on human femoral heads, the significantly reduced F\(_{\text{Pullout}}\) for screws tightened to 90\%T\(_{\text{Max}}\) compared to 80\%T\(_{\text{Max}}\) may be a result of yielding of trabecular bone structures. F\(_{\text{Pullout}}\) at 80\%T\(_{\text{Max}}\) tightening was also greater compared to F\(_{\text{Pullout}}\) at 70\%T\(_{\text{Max}}\), although not significantly, which could be due to sample size.

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ACKNOWLEDGEMENTS

This study is funded by NHMRC Project Grant ID 595933.
INTRODUCTION
Due to a paucity of paediatric cadaver specimens, and the unique injury and disease mechanisms of the paediatric spine, a well characterised animal model is needed for biomechanics research. The mature sheep is a well established model for the adult spine [1,2], but no equivalent exists for the paediatric population. The aim of this study was to develop mathematical relationships describing paediatric human and immature sheep vertebrae in terms of skeletal maturation, anatomical dimensions and mechanical behaviour.

METHODS
Spines from Merino sheep aged 2, 6, 12, 24 and 39 weeks (±12hr, all regions), and 3 years (±3m, cervical only) (N=36) were prepared. C1 & C2 synchondrosis ossification was identified radiographically, and human data obtained from literature. Vertebral dimensions (body height/depth, canal width/depth) were measured with calipers for C3, C4, T5, T6, L3 and L4 vertebrae, and similar human data was obtained from literature. The C4, T6 and L4 vertebral bodies were pre-conditioned and loaded to failure in axial compression (Instron 8874; 6 mm/min), and mechanical properties derived. Power-law curve fits were applied to the sheep and human data.

RESULTS AND DISCUSSION
C1 and C2 ossification centres were in similar locations for both species, but synchondrosis ossification occurred more rapidly in sheep, and in a different order (Figure 1).

Sheep vertebral dimensions and material properties increased with age according to power laws. For example, from 2 to 39 weeks, L4 body height increased from 18.2±0.9 mm to 33.3±1.9 mm (human L3 2yr: 16 mm; 18yr: 33 mm; [3]), while stiffness increased from 2.22±0.26 kN/mm to 8.57±1.97 kN/mm, apparent modulus 171±26 MPa to 740±152 MPa, ultimate load 2.29±0.66 kN to 8.66±1.60 kN (Figure 2) (human lumbar 20-39yr: 7.16±0.13 kN [4]), and apparent ultimate stress 9.6±2.7 MPa to 22.6±4.1 MPa (human lumbar 20-39yr: 6.3±0.1 MPa [4]).

Figure 2: Ultimate load for the C4, T6 and L4 vertebrae of immature sheep, as function of age.

CONCLUSIONS
Mathematical relationships derived from this unique dataset can be used to select immature sheep to model paediatric spine biomechanics, based on skeletal ossification, vertebral size and/or vertebral body mechanical behaviour. Future testing will add a 52 week age group, and characterise discs and trabecular bone with respect to microstructure and mechanics.

ACKNOWLEDGEMENTS
The authors acknowledge technical support from the Large Animal Research & Imaging Facility, SAHMRI. Claire Jones is supported by an NHMRC Early Career Research Fellowship.

REFERENCES
INTRODUCTION
Image guided failure assessment of bone has been employed to analyse trabecular mechanics during uniaxial compression [1, 2], screw pull-out [3], and screw push-in tests [4]. These studies have provided valuable insight into the failure mechanisms of bone under specific loading conditions. Work within our laboratory, however, has sought to better understand the interactions between bone and implant during screw placement. Previous work within our lab has demonstrated that for lag screws in cancellous bone, the stripping torque ($T_{\text{max}}$) can be predicted based on the torque at head contact [5]. The question remains, however “how tight is tight enough?”. The aim of this ongoing study was to perform time elapsed image acquisition during screw insertion for lag screws in human cancellous femoral head bone.

METHODS
Ten excised human femoral heads were used. Custom manufactured aluminium lag screws (outer diameter = 7.0mm), were inserted to head contact. The torque measured at head contact ($T_{HC}$) was used to predict stripping torque ($T_{\text{max}}$) [5]. Screws were incrementally tightened using a novel testing device within the micro-CT scanner [6]. Micro-CT scans (isotropic pixel size 17 µm) were performed at 6 time points from head contact to $T_{\text{max}}$. 2D and 3D images were reconstructed and visually inspected to identify trabecular deformation around the screw threads.

RESULTS AND DISCUSSION
Ten tests were successfully completed in conjunction with micro-CT image acquisition. Significant debris is evident at the direct bone-screw interface, most likely due to the absence of tapping prior to screw insertion. Preliminary visual image analysis suggests that the majority of deformation occurs after the 80% step between head contact and $T_{\text{max}}$. Future work will look to employ finite element analysis or digital volume correlation to quantify the peri-implant strains induced with increased tightening up to $T_{\text{max}}$. This influence the recommended tightening levels of lag screws in cancellous bone. There is likely an optimal tightening level, that can achieve sufficient inter-fragmentary compression (to allow fracture healing), without jeopardizing the structural integrity of the peri-implant bone. What this level is, however requires further investigations.

CONCLUSIONS
This study has demonstrated the ability to visualize peri-implant deformation during the tightening phase of screw insertion. An increased understanding of the effects of increased screw tightening at the micro-structural level may help to reduce the incidence of screw stripping during insertion.

ACKNOWLEDGEMENTS
This study was funded by the NHMRC Grant ID 595933.

REFERENCES
THE EFFECT OF SIX DEGREE OF FREEDOM LOADING ON THE IN-VITRO COMpressive RECOVERY PROPERTIES OF HUMAN LUMBAR SPINE SEGMENTS

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INTRODUCTION
The complex, direction-dependent, poro-viscoelastic properties of the intervertebral disc (disc) suggest that investigations of the six degree of freedom (6DOF) behavior should include recovery periods between test directions. Previous studies have subjected the disc to 6DOF loading without indicating if a recovery period in between tests was applied [1,2], which could introduce variation in mechanical properties between laboratories. To our knowledge, no studies have quantified the effects of sequential 6DOF loading on the consistency of the disc’s mechanical response. Therefore, the aim of this study was to determine the effect of different loading directions in 6DOF on the compressive recovery properties of the human discs, as evaluated by a reference compression test performed after each single DOF test.

METHODS
Fourteen intact functional spinal units (FSUs) were dissected from human lumbar spines (L1-2 x 5, L2-3 x 3, L3-4 x 4, L4-5 x 2, mean (SD) age 76.2 (11) years, Thompson grades 3 (N=5), 4 (N=6), 5 (N=3)). Each FSU was subjected to loading in 6DOF at four loading frequencies, under a 0.5 MPa compressive preload using a hexapod robot in a protease inhibited 37°C phosphate-buffered saline bath [4]. Initially, the disc was equilibrated for 14 hrs at a compressive preload of 0.1MPa. The baseline (control) compressive properties were then established by conducting a reference compression test at 1.1 MPa (haversine waveform, 0.1 Hz, 8 cycles). The 6DOF testing sequence was then commenced, each followed by a reference compression test. The loading frequency and 6DOF direction sequence were carefully chosen to theoretically minimise biphasic effects by conducting shear tests first, followed by bending and compression [4]. Creep recovery periods at 0.1 MPa were included between directions to allow for viscoelastic relaxation (5 mins for shears and 10 mins for bending/compression). A repeated measures ANOVA was performed on the outcome measures of average stiffness (entire final load-unload cycle), having a within-subjects factor of reference compression test. Post-hoc multiple comparisons using a Bonferroni correction on alpha were performed when significant main effects were present.

RESULTS AND DISCUSSION
The overall testing time for each FSU was 32 hours under temperature, hydration, osmotic and putrefaction controlled conditions. There were significant within-subjects effects between the compression tests for average stiffness (p<0.001). Significant post-hoc pairwise comparisons were found between the control and most other tests (Figure 1).

Figure 1: Mean (95% CI) stiffness for each sequential reference compression test. * denotes significant difference in comparison to control (*p<0.01, **p<0.001).

Loading directions have an impact on compressive recovery properties of the disc. Although a change in disc fluid volume was not expected to occur during the shear tests, a trend of increasing stiffness over time was observed (Figure 1). This was followed by a steep decline in stiffness after the first bending test, which correlates with an expected excursion of fluid due to the volume change caused by bending. A steady state response was observed for the remaining reference tests during the bending sequence.

CONCLUSION
Sequential 6DOF loading significantly affects the compressive recovery properties of the disc and must be considered in comprehensive mechanical testing.

REFERENCES
TOTAL HIP ARTHROPLASTY IMPLANT CONDITION ASSESSMENT THROUGH ACOUSTIC EMISSION MONITORING

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INTRODUCTION
Early diagnosis of total hip arthroplasty implant deterioration can save significant time and cost by allowing better management of revision surgeries. Acoustic Emission (AE) monitoring of THR implants uses passive ultrasonic receivers to provide insight into wear mechanisms by relating vibrations observed during dynamic patient motion to failure modes and identified clinical outcomes.

AIMS AND OBJECTIVES
This study aims to develop a clinical diagnostic tool by comparing acoustic measurements from in-vivo patient testing and in-vitro implant testing on a range of THR implants. The identification of acoustic indicators of implant failures includes audible squeaking of hard-on-hard bearing combinations, to provide insight into possible vibration sources. This study also seeks to determine the tribology of the THR articulation surfaces to provide insight into the underlying mechanics of implant squeaking.

METHODS
An array of four passive ultrasonic sensors is utilized for in-vivo AE monitoring of patients with squeaking implants to establish the frequency characteristics and the location of vibrations within the soft tissue of the patient. Implants components retrieved during subsequent revision surgery are then manipulated in-vitro to relate motion types to observed in-vivo signal characteristics. Patient testing was given ethical approval from the New Zealand Upper South A regional ethics committee under approval number URA/10/11/075.

High magnification Scanning-Electron Microscopy (SEM) examination of the surface of the femoral head and acetabular liner was undertaken to provide insight into the wear mechanisms.

RESULTS AND DISCUSSION
Results indicate that implant squeaking is a multi-factor problem. The fundamental frequency of THR squeaking is observed to vary with the applied load and implant type. In-vitro testing indicates squeaking only occurs when there is a loss of lubrication between the bearing components, indicating that point loads or stripe wear that break the thin-film lubrication layer may contribute to implant squeaking in-vivo.

Examination of the acoustic emission data for in-vivo patient testing and subsequent in-vitro testing of flexion motions at the primary bearing interface show a strong correlation, as seen in Figure 1. Conversely, relative motion between the femoral head and the femoral stem at the morse-taper produced frequency content beyond 20kHz, which did not correlate with the in-vivo patient test results. This result tends to indicate the morse-taper movement is not a significant problem in-vivo.

Moreover, detailed examination of the femoral head bearing surface indicates evidence of inter-granular failure of the ceramic material. The area of contact and wear pattern can be seen with the naked eye, where a dull area with a notably higher surface roughness can be seen due to localised surface degradation.

Figure 1: Representative acoustic emission profiles overlaid. For this patient, a strong correlation exists between in-vivo recordings and in-vitro testing of movement at the primary bearing interface.

CONCLUSIONS
Overall, implant squeaking is a complex, multi-variable problem. This study indicates that localised loss of thin-film lubrication can contribute to implant squeaking of ceramic-on-ceramic bearing combinations and the occurrence of inter-granular failure of the ceramic bearing material. Ongoing research is investigation the possibility of using inertial measurement units to augment the existing AE sensor technology to provide dynamic measurement of patient joint angles which can be correlated to the AE data.

ACKNOWLEDGEMENTS
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Body segment parameter estimation using Hatze’s geometric model

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INTRODUCTION

Estimating body segment parameters, such as mass, centroid, and moment of inertia, is necessary for performing dynamic analyses of human motion [1]. The accuracy of the results from such studies can be affected by the quality of this estimation, which historically uses one of two methods: regression models and geometric models. Regression models are distillations of large datasets of anthropometric measurements and body scans, and are valid only if the body shape of the subject falls within the bounds of the population studied to derive the regression model [2]. Geometric models are constructed with standard volumetric shapes sized using anthropometric measurements from each subject, and are therefore capable of estimating body segment parameters with greater flexibility [4,5].

In this study, a number of body segment parameter models are directly compared. Hatze’s model [1] is emphasised, as it is usually overlooked in the literature [3]. Deficiencies in these models are highlighted, with suggested improvements in their construction. It is suggested that greater emphasis be placed on body segment parameters for analysis of human motion.

METHODS

The Hatze model was reconstructed from the publication describing the model [1], the technical report deriving the mathematical equations [6], and the technical manual for its Fortran implementation [7]. The report and the manual describe the model at different times and have some divergent details; both contain typographical or mathematical errors that required careful analysis to correct.

Table 1: Calculated values of mass and centroid with the reimplementation of Hatze’s body segment parameter model compared to the reference implementation.

<table>
<thead>
<tr>
<th>Segment</th>
<th>Calc Hatze Err, %</th>
<th>Calc Hatze Err, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal-thoracic</td>
<td>18.487 ± 0.00</td>
<td>203 ± 0.00</td>
</tr>
<tr>
<td>Head-neck</td>
<td>5.187 ± 0.00</td>
<td>137 ± 0.00</td>
</tr>
<tr>
<td>Left shoulder</td>
<td>1.656 ± 1.84</td>
<td>151 ± 1.31</td>
</tr>
<tr>
<td>Left arm</td>
<td>2.315 ± 2.200</td>
<td>0.00 ± 131</td>
</tr>
<tr>
<td>Left forearm</td>
<td>1.157 ± 1.775</td>
<td>0.00 ± 112</td>
</tr>
<tr>
<td>Left hand</td>
<td>0.539 ± 0.542</td>
<td>0.00 ± 61</td>
</tr>
<tr>
<td>Right shoulder</td>
<td>2.105 ± 2.076</td>
<td>157 ± 0.63</td>
</tr>
<tr>
<td>Right arm</td>
<td>2.357 ± 2.362</td>
<td>0.00 ± 129</td>
</tr>
<tr>
<td>Right forearm</td>
<td>1.310 ± 1.345</td>
<td>0.00 ± 114</td>
</tr>
<tr>
<td>Right hand</td>
<td>0.524 ± 0.529</td>
<td>0.00 ± 63</td>
</tr>
<tr>
<td>Abdominal-pelvic</td>
<td>9.255 ± 2.43</td>
<td>0.00 ± 78</td>
</tr>
<tr>
<td>Left thigh</td>
<td>8.962 ± 8.938</td>
<td>0.00 ± 217</td>
</tr>
<tr>
<td>Left leg</td>
<td>3.994 ± 3.997</td>
<td>0.00 ± 186</td>
</tr>
<tr>
<td>Left foot</td>
<td>1.098 ± 1.098</td>
<td>0.00 ± 39</td>
</tr>
<tr>
<td>Right thigh</td>
<td>8.537 ± 2.155</td>
<td>0.00 ± 208</td>
</tr>
<tr>
<td>Right leg</td>
<td>4.088 ± 4.085</td>
<td>0.00 ± 194</td>
</tr>
<tr>
<td>Right foot</td>
<td>1.109 ± 1.109</td>
<td>0.00 ± 38</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The new implementation provides an accurate visualisation of the Hatze model for the first time (Figure 1). Despite careful attention, the results from the Hatze model contained some discrepancies with the reference implementation (Table 1). It is possible that the reference values also contain some erroneous results.

The choice of body model affects the calculation of the parameters; a more detailed model will require more time to take a larger number of measurements but will produce more accurate results. The choice of which to use will depend on the details of the overall study.

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INTRODUCTION
Musculoskeletal models (MSMs), embedding multi-body optimisation algorithms, are used to predict the joint kinematics and the forces transmitted by internal structures for clinical and research purposes. They are typically driven using skin-mounted marker trajectories measured with stereophotogrammetry, which are affected by the artefact movement of the markers relative to the underlying bone (soft tissue artefact: STA). The effects of the STA on the MSM model outputs, however, are still unclear. The aim of this study is to investigate the sensitivity to the STA of the muscle and joint forces estimated by a MSM selected among those implemented in OpenSim [1].

METHODS
Twenty-eight 8mm-diameter reflective markers were attached to the feet (8), shanks (8), thighs (8), and pelvis (4) of a healthy volunteer (male, age: 28, stature: 190 cm, mass: 82 kg). Marker trajectories (Vicon Ltd, Oxford, UK) and ground reaction forces (Bertec Co., Columbus, USA) were recorded during static posture and level walking at self-selected speed. Within OpenSim, a generic MSM [1] was scaled to match the subject’s anthropometry and used to determine the pose of the bone anatomical frames. Joint angles, moments, and muscle and joint forces were calculated using the OpenSim’s built-in tools, namely, inverse kinematics, inverse dynamics, static optimization and joint reaction analysis. The time-independent positions of the 28 markers have been assigned in the relevant anatomical frames, and their trajectories have then been calculated in the laboratory frame. These synthetic datasets have been used as error-free reference data. The patterns of corresponding computed reference muscle and joint forces were comparable to published patterns [2]. STAs at the feet, shanks, lateral femoral epicondyles and pelvis were modelled as sinusoidal functions of the gait-cycle percentage [3]. The STAs for the lateral-thigh markers were reconstructed as a linear function of hip flexion, abduction, rotation and knee flexion angle [4]. For each marker and spatial coordinate, the mean and standard deviation of the amplitude, frequency and phase of the first STA model and of the five constants embedded in the second STA model were taken from the literature [3,4], resulting in 288 variables. Latin Hypercube Sampling was used to generate 500 samples for each of the 288 stochastic variables, thus producing 500 STA realisations. These were added to the reference marker trajectories and the resulting artefact-affected trajectories were iteratively fed to the MSM. The appropriateness of the simulated STA realisations was verified for each bony segment by averaging the peaks of the magnitude of the STA computed for each of the segment markers over the 500 samples and comparing these values with the literature [5]. Muscle force ranges expressed in body weight (BW) were calculated as the peak difference between the 95th and 5th percentile of the output variability. To quantify the observed variability as a percentage of the observed quantities, the ranges were expressed as percent of the 95th percentile values. The sensitivity of joint contact forces was identically assessed.

RESULTS AND DISCUSSION
The average peak of the STAs were 40.8 ± 8.3 mm at the thigh and 11.9 ± 5.2 mm at the shank, consistently with the literature [5]. Joint angle variations were in the range 6–21° (highest values for the hip flexion) in agreement with [5]. Joint moments were in the range 0.05–0.48 Nm/kg (highest values for the hip flexion moment). The muscle force range reached 0.92 BW (36%) for the Soleus. Sensitivity of joint contact forces to STAs was joint-dependent showing variations of 1.01 BW (25%), 0.89 BW (24%) and 0.37 BW (7%) for the hip, knee and ankle respectively (Figure 1).

Figure 1: The hip, knee and ankle force variations.

CONCLUSIONS
The sensitivity of joint contact forces to STA is joint-dependent and increases from the ankle to the hip while preserving force patterns. More research is needed to minimize the STA effect, particularly at the hip.

ACKNOWLEDGEMENTS
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REFERENCES
A STATISTICAL MODEL TO PREDICT BONE CELL DIFFUSION PATTERNS IN SCAFFOLDS


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INTRODUCTION

This study is motivated by the challenges in (i) estimating bone cell diffusivity for scaffolds and (ii) how to model the impact of in vivo loadings on bone cell diffusion. The difficulty arises as these important parameters are often not determined experimentally and not found elsewhere. Furthermore, in vivo loading is hard to study experimentally. We present a novel computational framework to evaluate bone scaffold diffusivity, predict cell diffusion patterns and estimate the influence of in vivo loadings.

METHODS

An overall framework is presented in Figure 1. i) A porous PLLA scaffold was manufactured and tested with cell viability assays; ii) AlamarBlue cell viability assays and live/dead staining assays had been used to determine the cell viability of porous osteoblast-PLLA (poly(L-lactide)) constructs [1]; iii) support vector regression models estimated the scaffold’s diffusion coefficients in Fick’s second law, and the finite element method was used to solve the definite integral of Fick’s second law; iv) Fick’s second law was solved for a case whereby cells migrated under pressure.

RESULTS AND DISCUSSION

The cell pattern was able to be predicted at a global level including the fluctuating cell concentrations over 20 days. When the support vector model was increased to 100 training data sets we were also able to predict the finer spatial pattern better. Further improvements in resolution may only be possible with a finer mesh. Our results suggest that the statistical model is useful in practice and is an improvement over impractical experimental approaches or approximation based on theories of cell migration. We believe our approach compares favorably to others as not all findings could be transferred across studies, and as our approach finds suitable parameters directly from our own experiment data.

Our simulations showed that pressure is a dominant effect on cell concentration pattern. In regions where there was high hydrostatic pressure the cell concentration was low. Although Fick’s law is a simplification on cell diffusion and osteoblast cells may have other cell mechanics interactions that dictate their migration behavior, our results suggest the effect of pressure should not be ignored in scaffold design.

CONCLUSIONS

The proposed algorithm could be useful for estimating diffusion coefficient in Fick’s second law directly from the experimental data for a cell migration model. Loading the model under pressure in silico suggests we should not ignore the influence of pressure on cell migration.

ACKNOWLEDGEMENTS

The authors acknowledge a Bioengineering Masters scholarship for W Fok and FRDF funding to J Fernandez.

REFERENCES

INTRODUCTION
The annulus-endplate anchorage system performs a vital role in the disc, creating a strong structural link between the compliant annulus and the adjacent vertebrae. The endplate is thought to be a potentially vulnerable region in the disc under overload conditions [1], a recent clinical study indicating that its failure more frequently leads to disc herniation than annular rupture alone [2]. However, several structural aspects of this critical anchorage site remain unclear.

Employing an ovine disc model and high-resolution multi-scalar imaging techniques, the aim of this study was to explore the structure of the annulus-endplate junction and the fundamental principles that form the basis of its resistance to failure.

METHODS
Mature ovine motion segments were prepared and examined via differential interference contrast optical microscopy and scanning electron microscopy for comprehensive structural analysis at both the micro- and fibril-levels. Some samples were chemically-fixed in torsion to observe the junction in both its tensioned and relaxed states. To identify anchorage-related failure mechanisms, motion segments were loaded to failure in three different configurations – torsion, in-plane tension and axial tension. The latter corresponds to the loading mode that the structural elements in the posterior region experience when the disc is loaded in flexion. Decalcified samples were also tested to understand the mechanical role of the mineralized component. All testing was followed by detailed structural analysis to determine the mode of failure.

RESULTS AND DISCUSSION
Microscopically, anchorage in mature discs occurs via the penetration of annular fibre bundles through the relatively shallow depth of the cartilaginous endplate (most of which is calcified) down to the bone interface. Closer inspection revealed that the annular bundles split into multiple sub-bundles within the endplate. This branching morphology is thought to increase the interface area over which forces are distributed and thus strengthen the anchorage. Evidence of a fibril-based integration system was also found, with fibrils from the branched annular sub-bundles merging with those from both the cartilaginous endplate and vertebral bone, and in some cases, penetrating beyond the cement line (i.e. the endplate-bone interface) to a limited depth. Although short-ranged, this finer level of integration will also contribute to the anchorage’s resistance to failure.

Microscopic examination of the torsion-fixed samples showed that when tensioned, the annular fibres bend at the tidemark (i.e. the annulus-calcified endplate interface). When unloaded, the fibres display a fine crimp (waviness) above their endplate anchorage depth but not over it. These findings are consistent with an anchorage into a much stiffer matrix, namely the calcified cartilaginous endplate.

Structural analysis of the overloaded samples revealed two main modes of anchorage failure – failure at the tidemark and failure at the cement line. Samples tested in axial tension contained a higher frequency of anchorage failures compared to those tested in torsion and in-plane tension, especially at the tidemark. This is presumably due to axial tension (and so flexion) resulting in a greater degree of acute fibre bending at this soft-hard tissue interface, and is consistent with previous evidence indicating that flexion increases the risk of disc herniation involving endplate failure [3,4]. Following decalcification, failure occurred more frequently at the cement line, thus highlighting the significant role that calcification plays in reinforcing endplate-vertebral bone adhesion.

CONCLUSIONS
This study sheds new light on the structural mechanisms by which annulus-endplate anchorage is achieved, including micro-level branching, fibril-level integration and calcification. It also provides insights into the failure mechanisms of the anchorage system, highlighting its increased vulnerability in flexion.

ACKNOWLEDGEMENTS
The first author gratefully acknowledges the University of Auckland for the Doctoral Scholarship.

REFERENCES
A COMPUTATIONAL MODEL OF THE DUAL ACTION OF PTH IN POSTMENOPAUSAL OSTEOPOROSIS BASED ON INTRACELLULAR REGULATION OF OSTEOBLAST APOPTOSIS

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INTRODUCTION
Osteoporosis is a disease characterized by long-term bone loss that occurs when bone resorption exceeds bone formation in the remodelling process. Progress has been made in the formulation of computational models of bone remodelling in order to take into account major cell-cell signalling pathways and regulatory mechanisms. Most of these models regulate bone cell activity via receptor-ligand binding reactions [1]. Many hormones exhibit different release patterns, such as continuous or intermittent, which then modulate differential cell behaviours. In the bone literature, the most prominent example is the dual action of parathyroid hormone (PTH). It has been shown that a continuous administration of PTH leads to a catabolic effect on bone remodelling. On the other hand, daily subcutaneous injections of PTH constitute an effective anabolic treatment for osteoporosis. However, current computational models of bone remodelling are not able to distinguish between these two administration patterns. The purpose of our study was to develop a computational model of bone remodelling that takes into account the dual action of PTH. The model was built using human pharmacokinetics data, then calibrated and validated with data from postmenopausal osteoporosis (PMO).

METHODS
The mechanism implemented in the model accounts for the anabolic effect of intermittent PTH on bone remodelling via the reduction of the apoptosis rate of active osteoblasts (OB). This mechanism involves the runt-related transcription factor 2 (Runx2) and the cAMP response element-binding protein (CREB). Runx2 is a mediator for the transcription of survival genes, such as B-cell lymphoma 2 (Bcl-2) [2]. The action of PTH on OB apoptosis is modelled with a system of three ODEs describing the intracellular signalling components (Runx2, CREB, and Bcl-2) as a function of PTH [3]. Changes of Bcl-2 drive the reduction of OB apoptosis rate. This action is implemented in the model via a sigmoid \( f_{\text{max}} \) function. The effect of PTH on bone remodelling is described by coupling the intracellular model of OB apoptosis together with a bone cell population model [1] to compute changes over time of bone cell numbers and bone matrix fraction \( (f_{\text{bom}}) \). The latter quantities can be linked to bone turnover markers (BTM) and bone mineral density (BMD). To reproduce the change over time of PTH concentration in plasma under different dosing regimens, a pharmacokinetic (PK) model has been developed.

RESULTS AND DISCUSSION
PMO is simulated with a rate of bone loss equal to 0.65%/year (Figure 1, bottom). The daily PTH subcutaneous injections are reproduced using a step function calibrated according to the PK model. The basal PTH concentration is 3 pM; after the injection of 20 \( \mu \)g of PTH the plasma concentration increases 15-fold for about 1.5 hours until it reaches basal values again. The computed apoptosis rate follows the intermittent behaviour of PTH (Figure 1, top), with an overall reduction in the daily average compared to baseline. The model shows a 3% trabecular \( f_{\text{bom}} \) increase from baseline after the simulation of 2 years of treatment (Figure 1, bottom). This value is consistent with the BMD increase measured at the femur neck and distal radius [4, 5]. Simulation of 40 \( \mu \)g PTH injections leads to a higher \( f_{\text{bom}} \) gain compared to the 20 \( \mu \)g dose. These results indicate that the model is capable of reproducing a dose-dependent gain in bone volume.

Figure 1: (Top) Effect of intermittent PTH (20 \( \mu \)g/day) on OB apoptosis. (Bottom) \( f_{\text{bom}} \) change over time (% from baseline). Dashed line: baseline. Solid black line: PMO evolution (no treatment). Blue solid line: treatment with PTH (20 \( \mu \)g/day). Red solid line: treatment with PTH (40 \( \mu \)g/day).

CONCLUSIONS
By coupling the intracellular signalling of OB apoptosis and the bone modelling/remodelling process, our model can simulate the anabolic effect of intermittently administered PTH for PMO treatment. The simulated \( f_{\text{bom}} \) change over time is dose-dependent, and can be linked to BMD. Furthermore, simulation of a continuous administration pattern reproduces the catabolic effect of PTH on bone, used to analyse different disease scenarios (e.g. hyperparathyroidism).

REFERENCES
MECHANICAL PROPERTIES OF THIRD METACARPAL SUBCHONDRAL BONE IN THOROUGHBRED RACEHORSES

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INTRODUCTION
The fetlock joint is the most common site of musculoskeletal injury in racehorses. A large proportion of fetlock injuries, including fatal fractures, arise from the subchondral bone of the distal metacarpus. Characterizing the mechanical properties of distal metacarpus under loading conditions during racing is essential to understand the mechanism of such injuries. Previous studies measured mechanical properties of subchondral bone under a low rate loading [1]. In the present study, we applied displacements at a high rate that simulated the rate of loading which is applied to the metacarpal subchondral bone during galloping. We preserved cartilage in-situ. DIC image analysis was used to extract the displacement of subchondral bone layers from the overall displacement which was applied by the mechanical testing machine. The data was processed to find strains of subchondral bone below cartilage.

METHODS
Osteochondral plugs were extracted from medial condyles of the distal metacarpus of three racehorses. The experimental procedure was similar to a previous study [2]. Specimens were fixed to a base plate. Displacements were applied to the articular surface using a mechanical testing machine (Instron, 8874, UK) while specimens were imaged using a high-speed camera. Time to peak strain was chosen 0.05sec [3]. Magnitudes of applied displacements were determined based on preliminary tests to apply 60-70% of the yield strain of subchondral bone [1]. Recorded images of each specimen were processed to find the average displacement of bone at three different levels (Fig. 1). Overall strains of two regions of interests, subchondral bone (SB) and trabecular bone (TB), were calculated by dividing the relative displacement of the upper and lower bounds (lines 1 and 2 for SB and lines 2 and 3 for TB) to the thickness of each region.

Stresses were synchronized to the strains measured on images to find stress-strain curves of each region for each specimen. The slope of the linear portion of stress-strain curves were calculated as the elastic modulus of each region for each specimen. The areas enclosed by stress-strain curves were divided by the area beneath the loading curve to calculate the relative energy loss for each region of each specimen (Fig. 2).

RESULTS AND DISCUSSION
The elastic modulus of TB was significantly higher than that of SB in a paired comparison (Table 1). Relative energy loss by SB was significantly higher than the relative energy loss by TB.

Table 1: Mean±SD of elastic modulus and relative energy loss by two regions of interest (SB and TB).

<table>
<thead>
<tr>
<th>Mechanical Properties</th>
<th>ROI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastic modulus (GPa)</td>
<td>SB</td>
<td>3.11±1.69</td>
</tr>
<tr>
<td></td>
<td>TB</td>
<td>7.49±4.00</td>
</tr>
<tr>
<td>Relative Energy Loss (%)</td>
<td>SB</td>
<td>45.6±13.9</td>
</tr>
<tr>
<td></td>
<td>TB</td>
<td>20.4±8.2</td>
</tr>
</tbody>
</table>

CONCLUSIONS
Stiffness of SB was lower compared to TB. The difference may be due to a difference in density and/or bone volume fraction of each region, which will be investigated in future studies. In addition, the greater ability of SB to dissipate energy may be due to microdamage in SB which is common in medial metacarpal condyles of racehorses.

ACKNOWLEDGEMENTS
Racing Victoria Limited for funding this project.

REFERENCES
MICRON -224 associates with the severity of LDH and regulates disc progenitor cell between chondrogenic and fibrogenic differentiation

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INTRODUCTION

Lumbar disc herniation (LDH) is one of the major causes for back and sciatic pain that costs large medical expense. Accumulating evidence has indicated that microRNAs (miRNAs), a small non-coding RNA molecule, emerge in the plasma of LDH patients and the profile changes in different stages. However, the role of miRNA in the LDH patients remains unclear. Herein, we hypothesize that the profile of circulating miRNAs have a potential of early diagnostic effect in LDH and the miRNA in disc further associates to homeostasis.

METHODS

MicroRNA profiling was performed on plasma samples of 4 groups: LDH young group (n=8, average age is 31), LDH-old group (n=8, average age is 56), healthy control-young group (n=4, average age is 20), healthy control-old group (n=6, average age is 58). The miRNAs expression was further validated by quantitative real-time RT-PCR (qRT-PCR) and cellular level.

RESULTS AND DISCUSSION

Compared to healthy control, miRNAs in LDH group is considered to be significant with P-values<0.05 and the fold change values $\geq 2$ or $\leq 0.5$ (Fig 1, 2). In particular, 61 miRNAs are upregulated and 302 are downregulated in the LDH-young group compared to healthy control-young group. 46 upregulated miRNAs and 115 downregulated are identified in LDH-old group compared to healthy-control-old group. Interestingly, 12 upregulated and 71 downregulated miRNAs are significant both in the young compared groups and the old compared groups (Selected miRs in Tab1, 2). Among them, miR-224 is upregulated 4.04 fold in the young compared groups and 16.99 fold in the old compared groups (p=0.01 for both). It is further validated to be consistent with the trend by qRT-PCR that increases in the LDH patients (Fig. 3). With the enhanced expression of the miR-224 in the disc of LDH patients, disc progenitor cells (DPCs) undergo apoptosis and the chondrogenic differentiation is shifted to fibrogenic differentiation (Fig. 4). Additionally, miR-224 targets apoptosis inhibitor-5 (API5) to induce the apoptosis of DPCs.

Figure 1. The volcano plot of miRNAs between each group. (A) Volcano plot between LDH-young and CK-young. (B) Volcano plot between LDH-old and CK-old. The red dots represent the miRNAs with P-values<0.05 and fold change values $\geq 2$ or $\leq 0.5$.

Figure 2. Heat map and hierarchical clustering. Heat map shows the results of two-way hierarchical clustering of miRNAs and samples. Each row represents a miRNA and each column represents a sample. The miRNA clustering tree is shown on the left, and the sample clustering tree appears at the top. The color scale shown at the top illustrates the relative expression level of a miRNA in the certain slide: red, high relative expression level; green, low relative expression level.
ANZORS – CHINA 2
INTRODUCTION

No matter what the source, injectable biomaterials must meet several criteria to perform successfully in clinical applications. They must be biocompatible, or able to function in vivo without eliciting an intolerable response in the body either locally or systemically. Adequate mechanical properties are also an important criterion for biomaterials, especially those used in devices intended to replace or reinforce load-bearing skeletal structures. The aim of this study was to compare the properties of the strontium containing bioactive bone cement with those of polymethylmethacrylate (PMMA) clinically.

Strontium-containing hydroxyapatite (Sr-HA) bioactive bone cement consists of a filler blend of strontium-containing hydroxyapatite and a resin blend of bisphenol A diglycidylether methacrylate, triethylene glycol dimethacrylate, poly(ethylene glycol) methacrylate, and N, N-dimethyl-p-toluidine. Its properties, including setting temperature, mechanical strength, biocompatibility as well as osteoinduction, were compared with other cements in vitro and in vivo, followed with a pilot study of clinical trial. This study suggest that strontium delivered locally has the same effect; thus, the combination of strontium with HA in a injectable biomaterial or cement with a low setting temperature, adequate stiffness, and low viscosity makes this a good bioactive cement for vertebroplasty. The injectable biomaterials and their future development as well as clinical applications such as hip fractures, plastic surgery and dentistry will be discussed.
INTRODUCTION
Lactoferrin is a multifunctional glycoprotein with therapeutic potential in bone tissue engineering. In vitro, lactoferrin potently enhances osteoblast proliferation and differentiation, is anti-apoptotic and inhibits osteoclast formation and activity [1]. Furthermore, lactoferrin is antimicrobial and modulates the immune response [2].

The aim of this study was to assess the effect of lactoferrin on bone regeneration in vivo.

METHODS
Five-millimetre critical-sized defects were created over the right parietal bone in 60 Sprague-Dawley rats. These were randomised into 3 groups:

- Group 1 = Empty defects.
- Group 2 = Defects filled with collagen gels (3mg/mL).
- Group 3 = Defects filled with collagen gels containing bovine lactoferrin (10μg/gel).

The rats were sacrificed at 4 or 12 weeks, the calvaria excised and imaged by micro-CT (SkyScan 1172) with a 12μm voxel size. Cylindrical volumes of interest were created. New bone formation, tissue mineral density (TMD) and porosity were measured.

The release profile of lactoferrin from the collagen gels was assessed in vitro. Lactoferrin containing gels were incubated in PBS over a 48 hour period and conditioned PBS was collected at various time points. Lactoferrin concentrations in the conditioned PBS were quantified by ELISA (Bethyl Laboratories, USA).

RESULTS AND DISCUSSION
The percentage of new bone formation (BV/TV from the defect side compared to BV/TV for the intact contralateral parietal bone) was significantly higher in the group treated with the collagen gel containing lactoferrin (Group 3) at both 4 and 12 weeks post-surgery. Percentage bone healing for groups 1, 2 and 3 were 42.7±4.2%, 35.9±5.9% and 62.6±2.5%, respectively, at 4 weeks (p=0.0019); and 41.3±5.2%, 45.8±4.8% and 74.6±4.3%, respectively, at 12 weeks (p<0.0001) (Representative reconstructions demonstrated in Figure 1). There was no difference in TMD between groups, suggesting the new bone was of similar quality between treatments. Group 2 had a lower porosity compared to Group 3 at 4 weeks (p<0.05), although this became non-significant by week 12.

In vitro release studies demonstrated that lactoferrin was rapidly released from within the collagen gels, with the majority of lactoferrin fully released by the 6 hour time point.

CONCLUSIONS
This study demonstrated that a burst release of low dose lactoferrin significantly increased bone regeneration in a rat calvarial defect model. The profound effect of lactoferrin on bone regeneration has huge therapeutic potential.

ACKNOWLEDGEMENTS
The authors would like to acknowledge the support of the Health Research Council NZ, the MedTech Centre of Research Excellence, the Ministry for Business, Innovation and Employment NZ and the Auckland Medical Research Foundation. This research is also partially funded by EU FP7-'SkelGEN' under grant agreement n° [318553].

REFERENCES
KEYNOTE 5 – Associate Professor Thor Besier
THE MUSCULOSKELETAL ATLAS PROJECT (MAP): ORTHOPAEDIC APPLICATIONS

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INTRODUCTION
Computational models have tremendous potential for orthopaedic application, particularly for in silico testing of implants to assess and predict performance. However, the ability of musculoskeletal models to predict clinical outcome is dependent on capturing key anatomical features and describing appropriate loads and boundary conditions. Image-based subject-specific models of the musculoskeletal system are capable of accurately estimating in vivo joint loads and show promise for clinical use [1]. However, creating subject-specific models is time-consuming and requires high levels of expertise. Importantly, there is often a ‘disconnect’ between continuum-level finite element models used to investigate joint or soft tissue mechanics and the rigid body dynamic musculoskeletal models to estimate muscle forces. To address these issues, we have developed the Musculoskeletal Atlas Project (MAP), an anatomical and functional atlas of the musculoskeletal system. Our aim is to produce a tool to rapidly generate subject-specific models for computational modelling. Here we present several applications to assess and predict orthopaedic implant design.

METHODS
We created a python-based software platform (Fig 1, the MAP Client) to facilitate segmentation and meshing of musculoskeletal structures. Users specify their ‘workflow’ using a drag-and-drop interface and a simple plug-in architecture facilitates customisation and community engagement. Active Shape Models derived from large image datasets guide the segmentation or scale existing mesh templates to match experimental data [2]. The initial anatomical population was derived from 320 clinical CT scans (the Melbourne Femur Collection) and includes surface meshes of the major lower limb bones and muscles. The mesh fitting method deals with sparse data and ensures anatomically feasible solutions when scaling a template mesh to match markers from motion capture. The subject-specific meshes exported from the MAP Client can be re-meshed for mechanics simulations or used to create anatomically detailed OpenSim musculoskeletal models. Medical imaging data can be saved along with the resulting models in the MAP Database, which is built on the Physiome Repository (models.physiomeproject.org). The web-based MAP Database supports access control, version tracking, and facilitates annotation and searching via the MAP Query tool. Our long-term vision is to foster a community of MAP users to accelerate the clinical use of computational models.

RESULTS AND DISCUSSION
The MAP Client has successfully been implemented to generate custom modelling workflows for several orthopaedic applications. We will present several examples, including the assessment of femoral stems across a population of femurs, the design of custom acetabulum implants, and the assessment of surgical intervention for femoral acetabular impingement.

ACKNOWLEDGEMENTS
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REFERENCES
INTRODUCTION
In earlier studies, work from this lab has shown that the initial stages of cartilage degeneration involves de-structuring of the interconnected collagen fibril network, only detectable using specialized light microscopy or scanning electron microscopy (SEM) \(^1,2\). From this, it was later hypothesized that these initial micro-level fibrillar structural changes, are significant enough to alter the local tissue mechanical environment, such that the underlying bone responds by remodeling in the zone of calcified cartilage \(^3\). The aim of this study is to determine to what extent the almost nano-scale fibrillar level structural changes in early cartilage degeneration is mechanically significant at the tissue level.

METHODS
Ten bovine patellae, free from any signs of degeneration as determined using India ink staining were used in the first group. For the second group, ten patellae (from mature cow), washed with India ink to confirm mild-to-moderate degeneration in the distal-lateral quadrant, was used. A layer of radial zone cartilage was extracted from the distal-lateral quadrant of all 20 patellae. This cartilage was divided into 3 parts. One part was used to determine the transverse instantaneous stiffness by applying 100% strain at 10% strain per minute. Another part was equilibrated in 0.15M saline and subsequently in distilled water after which swelling potential (i.e. increase in surface area) was measured. The final part was used for micro- and ultra-structural analysis using a micro-tensile device combined with differential interference contrast (DIC) microscopy and SEM.

RESULTS
In comparison to the degenerate tissue samples, the healthy (intact) tissue exhibited a significantly (p=0.000) higher stiffness and lower swelling potential (See graph in Fig 1). SEM imaging confirmed the fibrillar level de-structuring that was present in the degenerate tissue. At the microscale when the tissue was stretched to 15% strain, the intact cartilage still maintained its tightly woven, densely-interconnected fibrillar structure (Fig 1A), while in the degenerate tissue there was fibrillar de-structuring and re-aggregation into significant bundles, leaving large vacuous spaces (Fig 1B).

DISCUSSION
That the mechanical effects described in the present study are due mostly to fibrillar level changes and not proteoglycan loss is supported by another study\(^4\) from our lab that validated the bovine model of early osteoarthritic tissue changes showing no significant Safranin O staining intensity (for PG content) between healthy cartilage tissue and those that were mild-to-moderately degenerate like the tissue used here.

Figure 1: Mechanical data from the testing of intact and degenerate tissue that end up lying along orthogonal axes of relatively large stiffnesses and swelling potential respectively. This divergence is explained by the fibrillar-level imaging of stretched tissue, revealing dense interconnectivity in the intact tissue (A), and a loss of such mechanical advantage in the degenerate tissue (B). [Both images, A and B, were taken at the same magnification and a collagen fibril is the width of about 100nm]

The association between the overall cohesiveness of the fibrillar network with tissue stiffness leads to believe that tissue interconnectivity is crucial for homogeneous transverse load redistribution.

CONCLUSIONS
This study confirms that the combinatory effects of reduced tensile strength and increased swelling potential is indicative of the tissue’s micro-mechanical state, in the earliest stages of osteoarthritic degeneration.

ACKNOWLEDGEMENTS
The authors would like to thank the Royal Society of New Zealand for the Marsden grant which funded this study.

REFERENCES
INTRODUCTION
The motivation of this study was to determine how the subtle degrees of mild through to severe degeneration might influence the joint tissue response to an impact load. The purpose of the investigation is to gain insight into the relationship between subtle tissue degeneration and its vulnerability to more severe damage.

METHODS
This study utilised the bovine patella model of osteoarthritis (OA). Four groups were chosen depending on the visible macro level of existing degeneration according to the Outerbridge scale [1]. These groups ranged from healthy cartilage, G0 through to severely damaged G3. Each sample was impacted with either 1.6 or 2.3J of potential energy. After the impact, 40µm cryo-sections were taken in the radial direction. The sections were examined using differential interference contrast microscopy (Nikon Eclipse 80i). A vulnerability score was found for each sample by categorizing the damage based on a method previously used [2]. The damage found can be broken down into: Type I; fissure in the surface layer extending no deeper than the transition region. Type II; fissure in the surface layer extending into the mid-deep zone. Type III; delamination of the cartilage at the calcified intersection. Type IV; damage to the sub-chondral bone. The vulnerability score was found by assigning point values to damage present based on their severity, 1 point for type I damage, 2 points for type II damage etc. A score of 10 indicates the presence of all types of damage, thus an extremely vulnerable sample. A score of 0 indicates the sample suffered no damage at all.

RESULTS AND DISCUSSION
The pre-existing morphology was examined in un-impacted samples. The surface layer of G0 samples is seen to be visibly smooth with little disruption. In G1 samples, the surface layer has small irregularities and the cartilage is more swollen. G2 samples have a highly irregular surface layer, with large clefs extending through the transition region. G3 samples do not have an effective surface layer at all, instead being covered by many fissures which extend past the transition region into the mid-deep zone. The thickness of the cartilage layer was seen to increase in thickness from G0 to G2, and decrease from G2 to G3. Overall, the mean peak stress was only significantly different when G3 samples are compared to the healthy G0 samples. The mean impact stress of G3 samples was 14.2% lower than G0 in 2.3J impacts. The only significant change in vulnerability found was for the G1 sample at 2.3J which were 79% more vulnerable.

Table 1 shows the mean vulnerability score of each 10 sample group. When compared to the control G0 samples, the only significant change in vulnerability was for the G1 samples at 2.3J which were 79% more vulnerable.

Table 1: Mean vulnerability score of each sample group

<table>
<thead>
<tr>
<th>Sample</th>
<th>G0</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6J</td>
<td>2.8±0.88</td>
<td>2.1±0.78</td>
<td>3.0±0.77</td>
<td>2.2±0.42</td>
</tr>
<tr>
<td>2.3J</td>
<td>2.3±1.07</td>
<td>5.2±1.24</td>
<td>3.9±0.85</td>
<td>4.1±1.02</td>
</tr>
</tbody>
</table>

The increase in vulnerability could be explained by the minor fibril destructuring found in the swollen G1 matrix, effectively decreasing the fluid pressure within the matrix. In a previous study it was postulated that water in the matrix is able to effectively dissipate energy around the tip of cracks, arresting the crack propagation [3]. This is because the water decreases the energy to a point where the stress occurring ahead of the crack tip is lower than the fracture stress of the matrix. If the matrix no longer has adequate fluid pressure between the fibrils, less energy will be dissipated.

CONCLUSION
This study shows that articular cartilage exhibiting the non-visible stages of osteoarthritis associated with tissue swelling has a mechanical response similar to that of healthy cartilage, yet is more susceptible to a high level of damage from impact induced injuries.

REFERENCES
Integrating spine biomechanics with $^{18}$F-NaF PET imaging to assess lumbar pain and bone remodelling

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INTRODUCTION

$^{18}$F-fluoride as a sodium salt ($^{18}$F-NaF) is a radiotracer that has only recently been used to evaluate back pain [1]. An $^{18}$F-NaF PET/CT scan for this purpose shows abnormal focal uptake in the $^{18}$F-PET images at an earlier stage of stress (fatigue) fracture than visibility of fracture in the CT image [2]. While previous studies demonstrate links between increased $^{18}$F uptake imaged during $^{18}$F-NaF PET, bone remodelling as a result of abnormal stress or fracture, and pain, none compare the location and magnitude of $^{18}$F uptake with the location and magnitude of stress distributions in the lower spine.

Since individual differences in spine shape influence bone stress distribution, we hypothesise that the overall pattern of $^{18}$F uptake and bone stress will be similar for a given individual. The primary aim of this study was the comparison in the lower spine vertebræ between the magnitude and spatial distribution of $^{18}$F uptake and Von Mises stress under subject-specific body weight load.

METHODS

$^{18}$F-NaF PET/CT co-registered anonymised scans of the lumbar region of twenty-nine patients had been acquired following self-reported chronic lumbar pain. A voxel-based continuum-level generic spine model was built from the Visible Human Male [3] image set and from this the subject-specific models were generated by host-mesh customisation [4] to each patient’s CT image geometry and applying subject-specific body weight loads. Linear elastic simulations were run to compute Von Mises stress (VMS) at element centroids, and $^{18}$F uptake values were interpolated from the PET scans to these centroids. Colocalisation of VMS and $^{18}$F was tested by the correlation of VMS and $^{18}$F magnitudes, and co-occurrence of ‘peak’ centroid subsets as defined by quantile thresholds.

RESULTS AND DISCUSSION

No significant correlation was found between $^{18}$F and VMS magnitude. Co-occurrence between peak $^{18}$F and VMS slightly higher than that expected for an independent-distribution case was found, when peak thresholds were defined using the 50th–100th percentile interval.

Patterns of $^{18}$F and VMS distribution were visually compared over all patients. Though overall, $^{18}$F uptake patterns were similar to that of VMS, small areas of high $^{18}$F uptake in some individual patients did not appear to relate to individual spine geometry. Of the individual differences in $^{18}$F and VMS which did relate to individual differences in spine geometry, intervertebral disc height appeared to be significant. This was particularly apparent within scoliotic individuals as disc height varies on either side of the curvature (Figure 1), with peak stresses occurring in regions of thinner disc.

Figure 1: Von Mises stress contours plotted for an individual with slight spine curvature in the coronal plane. Anterior view, with section through coronal plane.

CONCLUSIONS

The findings of this study support the proposition that peak bone stresses play a role in inducing $^{18}$F uptake, stemming from variations in geometry between and within individuals. This work may assist clinicians with identifying mechanical causes of high $^{18}$F uptake and inform treatment strategies.

ACKNOWLEDGEMENTS

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REFERENCES

ASSESSMENT OF CHANGES IN SPATIAL HETEROGENEITY OF BONE TISSUE MINERAL DENSITY IN THE HUMAN FEMUR MIDSHAFT WITH AGE USING MICRO-CT

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INTRODUCTION
Osteoporosis, a disease characterized by loss of bone quantity and quality, represents a growing burden to developing and developing nations around the globe. Over the last decades, good progress has been made to quantify age-related changes in vascular pores which drive loss of bone mass and consequently lead to weakening of the organ’s resistance to fracture. However, the effects of changes in bone quality with age haven’t been analyzed in full detail. Mineralization of the bone matrix has been suggested to be one of the most essential bone quality parameters directly affecting bone material properties in terms of brittleness. Purpose of our study is to investigate bone Tissue Mineral Density (TMD) distribution in human femurs across the lifespan.

METHODS
Midshaft sections from deceased patients between 20 and 95 years old and having no known disease directly affecting their skeleton were obtained from the Melbourne femur collection [1]. The bone samples and two HA phantoms have been microCT-scanned at a resolution of 13.4 µm and reconstructed. Furthermore, it has been demonstrated that healthy bone matrix can be described as a multiscale material composed of three elementary components, namely HA, collagen, and water [2], and that these components’ concentrations are linked by unique bilinear relationships across ages, organs, and species [3]. By considering the x-rays physics underlying computed tomography, the grey values of the CT images are translated into pixel-specific TMD values. Revisiting a morphological concept for porosity measurement [4], an in-house segmentation algorithm allows us to analyze the TMD distribution in the periosteal, intracortical, and endosteal regions, as well as in eight anatomical regions of the cross-sections.

RESULTS AND DISCUSSION
TMD is consequently plotted for the full images, as to compare the mineral density through sex and age groups. Moreover, a decrease from the periosteal region to the endosteal region is generally observed. The TMD in different anatomical compartments of the midshaft is investigated to demonstrate patterns of mineralization around the cross-sections. Due to the fact that TMD is measured throughout the cross-sections, beam hardening may play a role in biasing experimental results. In order to avoid and minimize the effects of beam hardening we developed a new method for embedding the bone specimen into a clay material with attenuation properties similar to bone.

CONCLUSIONS
Knowledge of changes of TMD together with vascular porosity with age at different anatomical sites of the femur cross section opens the discussion about the influence of biochemical changes and mechanical loading on bone morphology and tissue composition.

Figure 1: Subdivisions of the femur midshaft: Color lines refer to separation between regions. The quadrants are: A, anterior; AM, anteromedial; M, medial; PM, posteromedial; P, posterior; PL, posterolateral; L, lateral; AL, anterolateral.

ACKNOWLEDGEMENTS
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REFERENCES
AGE-RELATED SHAPE CHARACTERISTICS IN THE EQUINE FETLOCK JOINT
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INTRODUCTION
Catastrophic fracture to the equine metacarpophalangeal (fetlock) joint is common in racing Thoroughbred horses. [1] The location of condylar fractures has been shown to be consistent. [2] This suggests that the structural morphology of the bone could be an important factor in understanding fracture development. Quantitative data on the morphology of the equine third metacarpal bone (Mc3), in particular the features on the epiphysis, is sparse. Recently Alrtib suggested that a shallower sagittal ridge angle was associated with increased risk of condylar fracture. [3] The purpose of this study was to investigate the shape characteristics of a population of Thoroughbreds of known age, sex and racing history. We hypothesized that younger horses have a shallower ridge angle and lower ridge height.

METHODS
Clinical CT data (n=70) from the left and right Mc3 of 15 young (<4yo) and 20 old (>6yo) horses were obtained (SOMATOM Definition Flash, slice thickness 0.6mm), and bone segmentation performed (Figure 1, Stradwin). Three planes were taken from the segmented mesh describing the bone. A central mediolateral longitudinal section was taken through the axis of the shaft, and two further sections were taken in tilted dorsal and palmar planes which intersected the articular surface perpendicularly at 30° (palmar) and 20° (dorsal). [4, 5]

The angle of the sagittal ridge was calculated by measuring the angle between the best fit lines on either side of the sagittal ridge.

RESULTS AND DISCUSSION
We found a large variation in sagittal ridge angles (1SD~10°) (Figure 2) and there was no significant difference in sagittal ridge angle between the old and young cohorts (p=0.71; Figure 2). There was a trend of a smaller ridge angle at the palmar site (p=0.08), compared to the central and dorsal site. Although our hypothesis was not supported, there might still be geometrical differences in other regions of the bone due to ageing. We will investigate the total shape variation using a statistical shape model and determine whether age is a significant predictor of shape variation.

![Figure 1: Plane extracted from the 3D bone mesh, at 20° dorsal to the central ridge.](image)

Figure 2: Variance in sagittal ridge angles for young (Y) and old (O) horses at three locations, the central sagittal slice (C), 30° palmar to this (P) and 20° dorsal (D).

Given the range in sagittal ridge angle shown here, it will be important to understand if those with shallow ridge angles will be prone to increased stress and subsequent fracture, as suggested by Alrtib [3]. We will investigate this by creating finite element models to estimate cartilage and bone stress within this population.

CONCLUSIONS
Preliminary results did not show a between-group difference in sagittal ridge angle. The variance, coupled with the knowledge that shape variation affects stress distribution and risk of injury, [4] merits further research, including higher experimental power and construction of a robust statistical shape model.

ACKNOWLEDGEMENTS
The authors are grateful for the support of Auckland Bioengineering Institute and Equine Trust of New Zealand.

REFERENCES
A Principal Component Analysis of Shape, Stress and 18F-NaF Bio-markers in the Lower Spine

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INTRODUCTION
18F-NaF is a biomarker with a high affinity for sites of bone remodelling. It also has the ability to indicate sites of abnormal bone remodelling long before bone degeneration is visible. While its use in the clinic is routine for the lower lumbar there is no information regarding any relationship with spine shape and stress patterns. This information may allow for early detection of bone degeneration from knowledge of shape and stress. This study explores the statistical relationship between spinal shape, 18F-NaF imaging biomarkers, and bone stress in the lower spine to evaluate (i) which features of spine shape most influence stress and NaF uptake patterns; and (ii) develop a statistical model to predict NaF uptake and stress from knowledge of shape.

METHODS
Twenty-three CT and 18F-NaF PET [1,2] data sets of the lower vertebrae, obtained with ethics approval from Mercy Hospital, Auckland, New Zealand, were used for this population study. From the data set, 12 subjects presented with significant 18F-NaF uptake and 11 were classified as controls because they presented with insignificant or no 18F-NaF uptake. This experiment consisted of four parts for creating the population model and analyzing it; (i) host-mesh fitting [3], (ii) 18F-NaF extraction, (iii) principal component analysis (PCA) and (iv) partial least squares regression (PLSR). Host-mesh fitting used a least squares approximation to morph 3D generic spinal models to create consistent patient specific anatomical models. A Gaussian and K-D Tree approximation was used to extract key locations of 18F-NaF uptake and register it to the anatomical model. PCA was used to extract the modes of variation within a population model, and PLSR analyzed the modes to find correlating relations between spinal shape, 18F-NaF uptake and bone stress.

RESULTS AND DISCUSSION
Analysis of the population groups using PCA and PLSR, found that there were no significant differences due to age, gender or BMI. PCA revealed that shape could be represented by 3 key features (i) sacral tilt, (ii) spinal curvature, and (iii) intervertebral disc spacing. PLSR linked these spinal shape features and revealed that 18F-NaF uptake was most affected by sacral tilt, spinal curvature, and vertebral disc spacing, respectively in that order. Bone stress was most affected by sacral tilt, vertebral disc spacing, and spine curvature, respectively in that order. PLSR also found correlations between the locations of peak 18F-NaF uptake and peak bone stress.

CONCLUSIONS
This study analyzed PCA and PLSR models, which showed (i) linear correlations exist between shape and 18F-NaF/bone stress and (ii) both are mainly affected by sacral tilt. This indicates that a person’s spinal shape affects their bone stress, which in turn causes higher 18F-NaF uptake due to bone remodeling. This suggests that spinal shape may play a predictor role in the clinic for patient bone stress patterns and 18F-NaF identification.

ACKNOWLEDGEMENTS
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REFERENCES
ON THE USE OF COLOURED MARKERS TO ENHANCE KINECT V2 PERFORMANCE FOR FEET TRACKING

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INTRODUCTION

Kinect for Windows v2 (Kinect for Xbox One) has generated considerable interest in the biomechanical and clinical research community as a low cost, portable and marker-less motion capture device. The in-built Kinect v2 body tracking algorithm has yielded promising measurements of spatio-temporal variables, but lower limb joint angles during gait cannot be obtained reliably [1]. We propose a new approach based on the calculation of joint angles using OpenSim Inverse Kinematics (IK), thus accounting for anatomical constraints on joints. In a previous study testing the accuracy of Kinect v2 using Vicon [2], we found poor agreement between the two systems for sagittal-plane ankle angles. This finding is also supported by others [3]. Several reasons for this finding may be postulated including: 1) feet are extremities of the body, thus their orientations cannot be inferred from the position of any child joints; 2) feet have a smaller volume compared to other limbs, thus they are represented by fewer points in the Kinect v2 3D depth map; 3) suboptimal segmentation of the depth map can confuse the body algorithm in discerning if points pertain to the feet or to the floor, thus adding further inaccuracies in feet tracking.

The purpose of this study was to enhance Kinect v2 foot tracking performance using coloured markers. Our previous work demonstrated the feasibility of using manually-selected 3D points from Kinect v2 depth stream for assessing spinal curvature [4]. We want to extend and automatize this approach, dynamically tracking coloured markers attached to specific lower limb landmarks.

METHODS

An ad-hoc, real-time computer vision tracking algorithm was developed using both RGB and depth streams provided by Kinect v2 as input. Four Ø36 mm coloured markers were attached to the subject’s lower limbs. Each RGB video frame was processed using a HSV filter. The resulting images were searched for contours and the 2D location of each contour was mapped into the depth stream, thus facilitating calculation of the 3D coordinates of coloured markers. Markers positions were exported into a trace file, together with those of Kinect v2 skeleton joints. Lower limb joint angles in the sagittal plane were calculated using IK and then synchronized to those obtained using Vicon marker data. Bland-Altman analysis of agreement between Kinect v2 and Vicon joint angles was then carried out.

RESULTS AND DISCUSSION

Preliminary results based on a single trial are promising. With the addition of coloured markers, the agreement between Kinect v2- and Vicon-derived ankle flexion angles during a squat task is increased and more constant across the range of motion, compared to the use of Kinect v2 skeleton algorithm alone. Limits of agreement are reduced from about ±10° (using the skeleton algorithm alone [2], Figure 1a) to about ±4° (adding coloured markers, Figure 1b).

Figure 1: Bland-Altman plots for ankle flexion in the sagittal plane (a) without coloured markers and (b) with coloured markers.

CONCLUSIONS

An ad-hoc, real-time tracking algorithm for coloured markers was developed to enhance human joint tracking capabilities of Kinect v2. Preliminary results indicate good agreement with gold-standard measurements of kinematics (Vicon). The software tools developed will facilitate greater application of Kinect v2 as a low cost, portable motion tracking solution in biomechanics, health, multibody mechanics, gaming and animation. The developed software will be available for download on www.kinedge.net.

REFERENCES

PODIUM 6
INTRODUCTION
Bone tissue engineering offers an alternative solution to the traditional bone substitutions e.g. autografts and allografts. This approach may help resolve the availability and donor site morbidity during autologous bone graft harvesting procedures and the risk of disease transmission with the use of allografts. However, due to the poor mechanical and non-osseointductive properties of the currently available synthetic grafting materials such as ceramic and polymeric materials, the scaffolds with superior bone regeneration ability are required. Magnesium (Mg), as a potential additive, plays an essential role in skeletal development. Our pervious study also showed that a specific amount of Mg ions is able to promote new bone formation. Hence, our group has fabricated a hybrid porous scaffold made of polycaprolactone (PCL) and Mg micro-particles. This study aims to investigate the mechanical, in vitro and in vivo properties of the newly developed scaffold.

EXPERIMENTAL METHODS
The Mg/PCL scaffolds were prepared by incorporating 4.8% 150μm Mg granules into PCL using salt leaching technique. Silane coupling agent (TMSPM) was coated on the Mg granules in order to enhance the bonding between PCL and Mg. Compression test was conducted to study the mechanical property of the scaffolds. A 7-day stimulated body fluid (SBF) immersion test was conducted to test their bioactivity. After that, the surface composition was checked by energy dispersive x-ray spectroscopy (EDS). The cytocompatibility of the scaffolds was studied by direct culture of green fluorescent protein mouse osteoblasts (GFPOB). Finally, the in vivo response of the scaffolds was evaluated by rat model for 3 months. Giemsa staining was used to visualize the new bone formation.

RESULTS AND DISCUSSION
The compressive modulus of the Mg/PCL scaffold was doubled as compared to pure PCL scaffold, whereas increased 80% further after the TMSPM treatment (Figure 1). The result suggested the mechanical property of pure PCL scaffold could be significantly enhanced by incorporating Mg granules. The silane coupling treatment could further enhance its mechanical property. Calcium and phosphate deposition was detected on the hybrid scaffold but not on pure PCL scaffold after 7-day SBF immersion (data not shown). The apatite layer formation illustrated the enhancement of osteoconductivity of the PCL scaffold. Moreover, the hybrid scaffold was fully engulfed by living osteoblasts as compared to the pure PCL scaffold which indicated that the hybrid scaffold was able to enhance cell attachment and growth (Figure 2). This was possibly due to the effect of Mg ions release. In previous study, low level of Mg ions (i.e. 50ppm) was able to stimulate growth and differentiation of osteoblasts. Hence, this explained why more new bone formation was able to grow inside the hybrid scaffold than pure PCL scaffold during animal implantation (Figure 3). The interconnected porous scaffolds would allow fluid flow, thereby enhancing bony tissue in-growth. All these favourable observations may attribute to the bioactivity enhancement due to the consistent release of Mg ions. However, the release of Mg ions should be controlled carefully since bone loss will be resulted if too much ions are released.

CONCLUSION
This study demonstrates that the newly developed Mg/PCL scaffolds are able to enhance the mechanical property and inferior bioactivity of pure PCL scaffold so as to encourage bone formation and in-growth. TMSPM silane-coupling treatment can further enhance the mechanical property of the scaffolds. Hence, all these promising results have shown that the modified Mg/PCL hybrid porous scaffolds can be potentially applied in large bone defect fixation.

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AO Trauma Research Grant, Hong Kong Research Grant Council Competitive Earmarked Research Grant (#71891).
**INTRODUCTION**

Bone grafts are used clinically where bone loss exceeds the body’s healing capacity. The current gold standard bone graft is autologous bone harvested from the iliac crest. However, this method has unacceptable complication rates of up to 50%. In the search for viable alternatives, the biomaterial scaffold market is rapidly expanding and is estimated to be a $434B (USD) market by 2017.

In 2013, the FDA adverse events reporting system received 1,078,029 reports of device failure, suggesting that inadequate information is currently being obtained during the pre-clinical assessment of novel scaffolds. Given that the majority of adverse events are related to immune rejection, we feel a greater understanding of how these materials interact with the immune system is imperative. We feel that a focus on the macrophage response to be particularly important, namely whether the materials illicit a pro-inflammatory (M1), or an anti-inflammatory (M2) response. This should enable better predictions of immune acceptance, prior to in vivo studies.

**METHODS**

A selection of novel biomaterials (aligned and random fibre electrospun PCL scaffolds, solvent-free fabricated PLA and PETG scaffolds, PHB-HV and GG/HA) were exposed to the human monocyte THP-1 cell-line. Controls included THP-1 cells cultured alone (-ve) or THP-1 cells cultured with addition of PMA (+ve). The gene expression levels of pro-inflammatory (M1) and anti-inflammatory (M2) cytokines following 1, 3 and 7 days culture were assessed by real-time PCR. In addition, protein secretion levels were assessed by ELISA. The screen evaluated ten cytokines related to M1/M2 response (Table 1).

**RESULTS AND DISCUSSION**

Following 24hrs culture with THP-1 cells, the PMA (+ve) control increased IL-1β expression >150-fold compared to -ve control. The PLA and PETG scaffolds induced >1000 and >175 times the expression of IL-1β compared to control, respectively. These expression levels remained significantly high following 72hrs of culture (Figure 1). The gene expression profile of other pro-inflammatory (M1) cytokines, such as TNFα, IL-6 and IL-8, were similar to that of IL-1β.

Expression levels of the anti-inflammatory cytokine IL-10 were significantly higher in cells exposed to the PLA scaffold on day 1 and the PETG scaffold on day 3 (5- and 6-fold, respectively); however, these were mild in comparison to the expression of the M1 cytokines. Gene expression profiles of other M2 cytokines IL-1α and TGFβ1 were similar to that of IL-10. IL-4 and IL-13 were not expressed in any samples.

The ELISA results largely mimicked the gene expression data, with the positive control, and the PLA and PETG scaffolds having significantly higher concentrations of IL-1β at both 24hr and 72hr time points (Figure 1). At the 24 hour time point, concentrations were 84.1, 134.4 and 45.6pg/ml, respectively, compared to control.

![Figure 1: Gene expression and protein secretion for the IL-1β (M1) cytokine.](image)

**CONCLUSIONS**

These results indicate that the PLA and PETG scaffolds produced cytokine profiles indicative of a chronic immune response. Previous cytocompatibility assays with these scaffolds suggested that they were good candidates for further study, with in vivo experiments planned; however, our immunogenicity screen identified significant levels of M1 activity, which prevented this further in vivo evaluation.

Given PLA and PETG are readily used in a clinical setting; this is likely a scaffold specific reaction, not a material specific reaction. Therefore, alterations to the fabrication process and/or the scaffold specific form (surface texture, pore size etc.) may alleviate this detrimental response.

Overall, this pre-clinical screen has the potential to prevent unnecessary animal use, therefore reducing experimental resources and enabling a safer transition from bench to bedside for novel bone graft substitutes.

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**REFERENCES**

IN VITRO AND IN VIVO EVALUATION OF PHB-HV AS A POTENTIAL SCAFFOLD FOR BONE REGENERATION

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INTRODUCTION

Bone tissue engineering requires the presence of a biocompatible scaffold that supports cell growth and enhances the native repair process. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB-HV) is a biodegradable 3D scaffold with 88.1±0.3% porosity and pore size of 163.5±0.1µm. Previous studies demonstrated the potential of PHB-HV to act as a scaffold in spinal cord repair [1]. The aim of this study was to evaluate PHB-HV as a scaffold for bone regeneration both in vitro and in vivo.

METHODS

PHB-HV scaffolds were manufactured through an emulsion freezing/freeze-drying technique. For cytocompatibility assays, primary rat and human osteoblasts were seeded onto the surface of PHB-HV scaffolds at a density of 2.5x10⁴ cells/well within 24 well tissue culture plates and cultured for 21 days. Assessment of cell viability was performed using the alamarBlue® assay (Invitrogen), accompanied by Live/Dead® Cell Viability assay (Life Technologies). Migration of cells through the scaffold was assessed by DAPI staining and fluorescent imaging across a transverse plane. For in vivo evaluation, critical-sized defects (5-mm diameter) were created in the right parietal bone of 40 male adult Sprague-Dawley rats. The rats were randomized into two groups: Group 1 – Defects left empty; Group 2 – Defects filled with PHB-HV scaffolds (5-mm diameter). The rats were sacrificed at 4 or 12 weeks post-operatively and the calvaria were imaged by μCT (Skyscan 1172) with a 12µm voxel size. Cylindrical volumes of interest were created and the dataset were analysed using CTAn software (Bruker μCT, Belgium). The percentage healing was calculated with reference to the contralateral side.

RESULTS AND DISCUSSION

Over the 21-day in vitro culture period, there was a significant increase in the number of viable rat and human osteoblasts cultured on the PHB-HV scaffolds (both p<0.0001). Live/Dead® and DAPI staining demonstrated viable osteoblasts colonised the surface of the 3D scaffold and began migrating through the porous scaffold (Figure 1).

In vivo, implantation with PHB-HV into rat calvarial defects did not significantly improve the degree of bone regeneration compared with empty defects.

Figure 1: a) Viable rat osteoblasts on the surface of a PHB-HV scaffold, 21 days post seeding; b) Cross sectional image of DAPI stained osteoblasts migrating through the PHB-HV scaffold, 14 days post seeding

CONCLUSIONS

PHB-HV is a naturally derived scaffold that supports the growth of primary bone cells. However, as an acellular, standalone bone graft alternative it does not improve in vivo bone regeneration in a calvarial defect. PHB-HV may therefore be better suited as a biocompatible and biodegradable scaffold that can deliver mesenchymal stem cells and/or growth factors to defect sites to encourage local bone regeneration.

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REFERENCES

INTRODUCTION
The repair and regeneration of large bone defects and non-union bone fractures has remained a major clinical challenge for orthopaedic surgeons. However, the limitations including immune-mediated rejection, senescence-induced genetic instability or loss of function, and limited cell survival hinder the adoption of direct transplant of MSCs in clinical applications.

In this study, by using the nanoparticles (exosomes) secreted by adipose tissue-derived MSCs (ASCs) (ASC-EXO), we circumvent the drawbacks (e.g. immune-mediated rejection, senescence-induced genetic instability or loss of function of MSCs) of the direct transplantation and develop a cell-free therapeutics to promoting bone healing and/or regeneration.

METHODS
First, ASC-derived conditioned medium (ASC-CM) or tumour necrosis factor-alpha (TNF-α) pre-treated ASCs-derived conditioned medium (TNF-ASC-CM) were used to test their ability to promote the proliferation, mobilization and osteogenic differentiation of human primary osteoblasts (HOBs). Second, the exosomes were depleted from ASC-CM or TNF-ASC-CM by ultracentrifuge of 100,000g, and then the exosome-depleted ASC-CM or TNF-ASC-CM were used for testing the roles of exosomes in ASC-CM or TNF-ASC-CM induced proliferation, mobilization and osteogenic differentiation of HOBs. Third, the exosomes isolated from ASC-CM or TNF-ASC-CM were characterized by Nanosight and western blot to determine the exosome size and molecular markers (CD9 and CD81), respectively. Finally, the protein component (Wnt-3a) in exosomes was analyzed and its function in inducing osteogenic differentiation in HOBs was further studied.

RESULTS AND DISCUSSION
1. ASC-CM (ASC-EXO) promoted the proliferation and mobilization of HOBs, and this effect is further potentiated by TNF-α pre-conditioning on ASCs.

Compared to the control group (serum free medium), ASC-CM significantly enhanced the proliferation of HOBs after they were cultured for 3 and 7 days, and this effect was further increased by the pre-conditioning with TNF-α (10ng/ml) on ASCs for 3 days. The similar phenomenon was observed for cell migration, and more HOBs were mobilized from the Matrigel in the group where HOBs were grown in ASC-CM and TNF-ASC-CM at 18 hours of culturing (Figure 1B). ASC-derived conditioned medium (ASC-CM) promoted the osteogenic differentiation (osteogenic gene expression and 2. The removal of ASC-EXO from the ASC-CM largely diminished its effect on the proliferation, migration and osteogenic differentiation in HOBs.

We depleted the exosomes by centrifuging the medium, and the exosomes-depleted ASC-CM or TNF-ASC-CM were then used to compare with their effects of ASC-CM or TNF-ASC-CM on proliferation, migration and differentiation. It was found that the depletion of exosomes from the medium significantly decreased the effects of ASC-CM or TNF-ASC-CM on cell proliferation, migration and osteogenic gene expression of HOBs.

3. Characterization of exosomes in ASC-CM or TNF-ASC-CM.

ASC-CM and TNF-ASC-CM were used for the characterization of exosome nanoparticles by Nanosight. ASC-CM and TNF-ASC-CM contained a large amount of nanoparticles with an average sized of 130nm. In addition, the exosome pellets purified from ASC-CM and TNF-ASC-CM expressed the typical markers of exosomes (CD9 and CD81).

4. TNF-α potentiated the function of ASC-EXO partially through a mechanism of increasing Wnt-3a content in ASC-EXO.

We further analysed the components of exosomes. It was found that the exosomes purified from the conditioned medium by TNF-α preconditioned ASCs had higher expression of wnt-3a than that in the conditioned medium from ASCs without TNF-α preconditioning. After we added IWR-1 (Wnt signalling inhibitor) into control medium, ASCCM or TNF-ASC-CM to culture HOBs, it was found that the presence of IWR-1 only significantly decreased the levels of Runx-2, osteopontin and bone sialoprotein gene expression in HOBs when they were cultured in TNF-ASC-CM, but not for control or ASC-CM.

CONCLUSIONS
The results demonstrated that ASC derived exosomes promote the proliferation, migration and osteogenic gene expression of human primary osteoblasts (HOBs) in vitro, which is potentiated by preconditioning ASCs with one major inflammatory factor (TNF-α), suggesting that ASC-derived exosomes might offer a promising approach to replace direct stem cell transplantation for bone repair and regeneration.
SYSTEMATIC MAPPING OF PROXIMAL TIBIA SUBCHONDRAL BONE MICROARCHITECTURE IN END-STAGE KNEE OSTEOARTHRITIS

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INTRODUCTION
In knee osteoarthritis (OA), changes to the proximal tibia subchondral bone microarchitecture are suggested to play an important role in initiation and progression of the pathology [1, 2]. However, the characterisation of region-specific variations within the knee, in the microarchitecture of the subchondral trabecular bone (STB) and thickness and porosity of the subchondral bone plate (SBP), is still limited.

The aims of this ongoing study in end-stage knee-OA patients are (1) to develop an analysis method that enables a systematic mapping for examining regional variations in the STB microarchitecture and the SBP thickness and porosity; (2) to explore possible region-specific associations between the STB microarchitecture and SBP thickness and porosity.

METHODS
Patients: Thirteen entire tibial plateaus were retrieved from patients with end-stage knee OA who underwent total knee replacement surgery (age 66±8 years, mass 95±20 kg).

Micro-CT Analysis: Entire tibial plateaus were scanned with micro-CT (17 µm/pixel, Skyscan 1076, Skyscan-Bruker, Belgium) and cross-section images of the entire tibial plateaus reconstructed. Within each tibial condyle (medial, lateral), 11 volumes of interest (VOIs, each 5 x 5 mm side, 5 mm height) were selected and in these, the STB and SBP were separated (software ‘CT Analyser’, Skyscan-Bruker). For the STB VOIs, the following 3D microarchitectural parameters were analysed: bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, µm), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, µm), structure model index (SMI). For the SBP VOIs, the 3D subchondral plate thickness (Pl.Th, µm) and plate porosity (Pl.Por, %) were calculated.

Statistics: In each condyle, differences in microarchitecture among the 11 subvolumes were tested by using repeated measures ANOVA followed by a Bonferroni post hoc test. Associations between STB microarchitecture, plate thickness and plate porosity were investigated using Pearson’s correlations. Significance levels were set to $p = 0.05$.

RESULTS AND DISCUSSION
The VOI exhibiting the highest BV/TV values was located centrally in the medial condyle and inner-posteriorly in the lateral condyle (BV/TV= 49±13% and 37±11% respectively, $p<0.05$ compared to average over 11 VOIs) (Fig. 1.a). In the medial condyle, the most posterior VOI exhibited the lowest BV/TV (28±13%, Fig. 1.a) and Pl.Th (388±140 µm, Fig.1b) compared with all other regions ($p< 0.05$). In the lateral condyle, the outer-anterior VOIs showed lower BV/TV and Pl.Th than VOIs located inner-posteriorly ($p< 0.05$). A positive correlation was found for ‘BV/TV vs. Pl.Th’ ($r= 0.66$, $p< 0.01$), whereas negative correlations were found for ‘Pl.Por vs. Pl.Th’ ($r= -0.60$, $p= 0.01$) and ‘BV/TV vs. Pl.Por’ ($r= -0.25$, $p< 0.01$).

CONCLUSIONS
The developed method enabled a mapping of the bone microarchitecture of entire tibial plateaus at micrometre resolution without multiple coring. Significant regional variations in bone microarchitectural parameters and correlations among them were found. Peak values of trabecular bone volume and plate thickness were found centrally and inner-posteriorly in the medial and lateral condyles respectively. As the subchondral bone is a mechanical shock-absorber, these variations could potentially reflect adaptations to the loading history of the joint [3].

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Figure 1: 3D micro-CT images of an entire right tibial plateau, top view (17 µm pixel size), with regional distribution of (a) STB BV/TV and (b) SBP Pl.Th within the tibial condyles. M = medial, L = lateral and P = posterior aspect.
### ANZORS 21st Annual Scientific Meeting

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