

Free Paper Session III — Basic Science I

3.1

Biological and Clinical Evaluation of Intra-operative Retention of Autologous Chondrocytes on Type I/II Collagen Scaffold (Ortho-ACI) 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. The study was designed to develop a more cost-effective method of this effective technique.

Methods: Cells were seeded onto the collagen scaffold and retention measured by confocal microscopy. Molecular markers of chondrocyte lineage were also compared against cells seeded for 4 days (current MACI). Patients were then recruited to receive the modified treatment. Cartilage repair was assessed by arthroscopy or magnetic resonance imaging (MRI) and graded. Repair outcome was correlated with variables such as patient age, graft size, and location.

Results: Collagen scaffolds retained 79% of cells after 7 minutes, increasing to 99% at 90 minutes. Molecular profile of chondrocytes seeded onto scaffolds for 20 minutes was more consistent with primary chondrocytes than those cultured for 4 days. Fifteen patients received treatment (25 grafts). Good / excellent repair was noted in 100% of grafts at a mean follow-up period of 25 months (MRI, n=5). Good / excellent repair was noted in 83% of grafts at a mean follow-up period of 17 months (arthroscopy, n=24). No associations were found between repair outcome and patient variables.

Conclusions: The Ortho-ACI technique involves seeding chondrocytes onto collagen scaffolds in theatre, a more cost-effective practice than normal MACI. Findings from in-vitro studies and case series of Ortho-ACI provide preliminary evidence that it is a safe, effective, and cost-effective MACI procedure.

3.2

Human Plasma Chemerin Level is not Associated with the Severity of Knee Osteoarthritis

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Objective: Adipokine-induced inflammation plays roles in the pathogenesis of human osteoarthritis (OA). Chemerin is a novel chemoattractant adipokine associated with metabolic syndrome and inflammation. This study aimed to investigate whether human plasma chemerin is associated with the severity of knee OA.

Methods: A total of 74 patients with knee OA were prospectively enrolled. The severity of OA was judged according to the Kellgren-Lawrence (KL) grading system. Patients' age, sex, body mass index (BMI), haemoglobin A1c (HbA1c) level, and lipid profile were documented. Plasma chemerin was detected by enzyme-linked immunosorbent assay.

Results: A total of 54 female and 20 male patients with a mean age of 60.9 years were included. Their mean (\pm standard deviation) triglyceride, low-density lipoprotein, high-density lipoprotein, cholesterol, and HbA1c levels was 1.58 ± 1.00 nmol/L, 3.04 ± 0.77 nmol/L, 1.50 ± 0.44 nmol/L, 5.25 ± 0.83 nmol/L, and 5.82 ± 0.65 mmol/L, respectively. The mean plasma chemerin level of patients with KL grade 2, grade 3, and grade 4 was 57.77 ± 16.80 (n=28), 62.93 ± 20.23 (n=27) and 60.84 ± 14.43 (n=19), respectively. The one-way analysis of variance showed no significant differences among these groups (p=0.413). The bivariate correlation analysis showed that chemerin was correlated with BMI (p=0.007, r=0.310) and triglyceride level (p=0.034, r=0.247).

Conclusions: Human plasma chemerin level is not associated with the severity of knee OA but is correlated with BMI and triglyceride levels.

3.3

Fatty Acid Binding Protein 4 as a Biomarker in Knee Osteoarthritis

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3.4

Fatty Acid Binding Protein 4 Initiates Cartilage Degeneration through Inducing the Cytokine Expressions of Macrophage — An In-vitro Study

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Introduction: Adipokine-induced inflammation plays roles in the pathogenesis of osteoarthritis (OA). Fatty acid binding protein 4 (FABP-4) is a novel adipokine that has been found to influence both metabolic and inflammatory pathways via the interaction with macrophage.

Methods: Murine macrophage (RAW 264.7) and primary murine chondrocyte (isolated from C57/Bl6 mice) were cultured. In all, 5 concentrations of murine FABP-4 (0, 16, 80, 400, and 2000 ng/mL) were supplemented to the macrophage culture medium and interacted for 24 hours. The conditioned medium was subsequently incubated with chondrocyte for another 24 hours. Gene expressions of cytokines interleukin (IL)-1 β , IL-6, and tumour necrosis factor- α (TNF- α) of macrophage, and matrix metalloproteinase (MMP)-2, MMP-3, and MMP-13 of chondrocytes were detected using real-time polymerase chain reaction.

Results: The mRNA expressions of all targets showed an increasing trend with the rise of FABP-4 concentration, with the expression folds of control (0 ng/mL FABP-4) being 1.62, 1.87, 2.80, 9.15 for IL-1 β ; 2.62, 4.25, 12.99, 83.71 for IL-6; 1.84, 2.07, 2.35, 2.27 for TNF- α ; 0.88, 1.07, 1.40, 2.13 for MMP-2; 1.15, 1.57, 1.75, 2.21 for MMP-3; and 1.03, 1.66, 1.73, 4.63 for MMP-13 under the concentrations of 16, 80, 400 and 2000 ng/mL FABP-4, respectively.

Conclusions: The FABP-4 initiates cartilage degeneration through inducing the cytokine expressions of macrophage.

3.5

Subchondral Trabecular Rod Loss and Trabecular Plate Stiffening in Patients with Knee Osteoarthritis

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Treatment with antiresorptive drugs enhances subchondral bone quality and attenuates cartilage erosions in osteoarthritis (OA), suggesting a role of subchondral bone loss in OA pathogenesis. We aimed to evaluate trabecular plate and rod microstructural changes in OA subchondral bone.

Tibial plateaus collected from knee OA patients (n=102) during arthroplasty and from cadaver donors (n=25) were scanned using micro-computed tomography. Five subchondral bone subregions (medial, central, lateral, anterior, and posterior) extracted from both medial and lateral condyles were subjected to individual trabecula segmentation and micro-finite element analysis. The specimens were then evaluated using histology.

In lateral, anterior, and posterior subregions on lateral condyle, Osteoarthritis Research Society International (OARSI) score, bone volume fraction (BV/TV), trabecular plate number and volume fraction (pBV/TV), and elastic moduli did not differ between groups. Significantly lower trabecular rod number and volume, increased rod thickness and length, increased trabecular plate thickness and surface and axial volume fraction, lower plate-plate, plate-rod and rod-rod junction density were detected in OA group. These results suggest that subchondral trabecular plate and rod changes may precede OA changes in cartilage. These changes occurred similarly in all subregions on medial condyle and medial and central subregions on lateral condyle, excepted that OARSI score, BV/TV, pBV/TV, and elastic moduli were higher in OA group.

Subchondral bone in human OA knees is characterised by trabecular rod loss and trabecular plate stiffening. Trabecular rod loss probably precedes plate stiffening, and thus may play an important role in OA pathogenesis.

3.6

Exacerbated Structural Impairments of Subchondral Bone and Articular Cartilage in Knee Osteoarthritis Patients with Hypertension**Y Chen,¹ WW Zhao,¹ FKL Leung,¹ X Cao,² W Lu¹**¹*Department of Orthopaedics and Traumatology, The University of Hong Kong, Hong Kong*²*Department of Orthopaedics, Johns Hopkins University, United States*

Subchondral bone loss increases cartilage damage in women following menopause, and in animal models with combined osteoarthritis (OA) and osteoporosis. Hypertension is associated with bone loss. Thus, we aimed to investigate microstructural changes in subchondral bone and cartilage in knee OA patients with hypertension.

Knee OA patients (n=102) undergoing arthroplasty were divided into non-hypertensive (n=48) and hypertension (n=54) groups according to hypertensive status. Tibial plateaus removed during operation were evaluated using micro-computed tomography (micro-CT), histology, and immunohistochemistry. Patients' clinical data were analysed.

The micro-CT analysis revealed that subchondral bone in hypertension group was lower in bone volume fraction (BV/TV), trabecular number (Tb.N), bone mineral density (BMD), and higher in structure model index (SMI) than non-hypertensive group. Significant associations were found between hypertensive status and BV/TV, Tb.N, and SMI after adjustment for age, gender, body mass index, and mechanical alignment. Histology showed higher Osteoarthritis Research Society International (OARSI) scores in hypertension group. Tartrate-resistant acid phosphatase staining detected larger number of osteoclasts in hypertension group. Immunohistochemistry revealed lower number of osterix + osteoprogenitors and osteocalcin + osteoblasts in hypertension group. Significant correlations were found between structural and remodelling parameters at subchondral bone and furthermore, between subchondral bone structural parameters and cartilage OARSI scores.

Subchondral bone and cartilage demonstrate exacerbated structural impairments in knee OA patients with hypertension. Changes in bone structure are associated with hypertensive status and, with cartilage degradation. Our results suggest that hypertension may induce exacerbated impairments in subchondral bone, and thus aggravate cartilage degradation in knee OA.

3.7

Computed Tomography-based Knee Joint Simulation

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Introduction: Knee joint simulation is essential for preoperative and postoperative analyses of lower limb deformity correction and total knee replacement. However, knees are always flexed in computed tomography (CT) scans and using X-ray images are inadequate for simulation. This study describes an innovative method to simulate the knee motion by simply using 1 set of CT scan.

Materials and Methods: A total of 5 CT scans with region of interest from femur to ankle were investigated. According to previous studies, the sagittal profiles of distal femur were divided into 2 portions, the posterior portion and the distal portion. Both portions of femoral condyles were fit with circular arcs. The curvatures were then used for simulating the gliding motion (distal curve) and the rotation motion (posterior curve) during the flexion and extension of knee. The knee gap distance was kept constant during the motion simulation. The coordinates of the hip centre were tracked during the motion simulation and further analysed in Matlab for locus analysis.

Results: Gliding movement contributed little ($<6^\circ/120^\circ$) on the total movement of femur flexion. Femur rotated around the posterior condylar axis in most time of flexion (about $115^\circ/120^\circ$). The rotational centre was more posterior during extension and more anterior during flexion (gliding moved the rotational centre anteriorly).

Discussion and Conclusion: The proposed method is efficient that only 1 CT scan is required for segmentation and simulation. However, patella movement simulation should be further included to facilitate a more complete knee motion simulation for knee biomechanics study.

3.8

Hypoxia Enhances Neurosphere Formation in Bone Marrow Stromal Cells via Epidermal Growth Factor Receptor Signalling

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Neural stem / progenitor cells form neurospheres when cultured in vitro. A subpopulation of bone marrow stromal cells is able to form neurospheres as well as differentiate into neurons and glia, making them an attractive source for autologous cell therapy. An understanding of what regulates the propensity for sphere formation, as well as the differentiation potential of these cells will enable one to enhance the yield and potency of neurospheres obtained from donor tissue. In this study, we demonstrate that hypoxia-mediated enhancement of epidermal growth factor receptor (EGFR) signalling is a mechanism by which neurosphere formation is increased. Hypoxia (1% O₂) leads to an upregulation in EGFR protein expression in bone marrow stromal cells, and subsequently an increase in the size and number of neurospheres as compared to normoxia controls. Subsequently, cells demonstrate an increased sensitivity to exogenous EGF. These effects are attenuated with the use of the EGFR inhibitor Erlotinib. Importantly, hypoxia did not affect the multipotency of the neurospheres, as evidenced by their ability to form cells of neuronal and glial lineages. These data suggest that targeting the EGFR pathway is an approach to enriching for neurosphere formation from patient tissue.

3.9

Strategies Developed for Enhancement of Enthesis Repair in a Rat Rotator Cuff Injury Model**JK Xu, JL Wang, SC Fu, YW Lee, KW Ho, PSH Yung, KM Chan, L Qin***Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong*

Introduction: We aimed to identify the specific cell source that contributes to better healing of rotator cuff (RC) and to investigate the effects of newly developed strategies, including tendon-derived stem cells (TDSCs) cell sheet and magnesium (Mg²⁺)-treated TDSCs cell sheet.

Materials and Methods: The spatial and temporal distributions of stem cells were evaluated during the natural healing of repaired RC in iododeoxyuridine (IdU) pretreated rats. Cell sheets were fabricated by incubating TDSCs in culture medium supplemented with ascorbic acid (25 μM), with or without addition of Mg²⁺ (10 mM) for 3 weeks. Then cell sheets were placed right between the torn end of supraspinatus tendon and the bleeding bone surface with original entheses removed. Animals receiving surgical repair only served as blank control. Haematoxylin and eosin staining and Safranin O staining were used to evaluate healing outcome.

Results: Although there were significantly increased (IdU+/CD18+) cells in the injured site at week 2 post-surgery, only scar tissues formed at the interface of blank repair animals. Both types of cell sheets showed beneficial effects in preventing fibrovascular formation at week 4 and week 8, at least partially attributing to the antiapoptotic effects of cell sheets. At week 16, significantly enhanced formation of fibrocartilage was observed in Mg²⁺-treated cell sheet group compared with cell sheet without Mg²⁺ treatment or blank repair group.

Discussion and Conclusion: The CD18-TDSCs are essential for the regeneration of entheses. The TDSC cell sheet pretreated with Mg²⁺ shows superior effect in the enhancement of RC repair.

3.10

'Proteoglycan Profiling' of Lumbar Disc Displacement in Humans: Novel Imaging Biomarkers Utilising T1rho Magnetic Resonance Imaging**H Pang,¹ ESK Hui,² JPY Cheung,¹ KDK Luk,¹ KMC Cheung,¹ D Samartzis¹***¹Department of Orthopaedics and Traumatology, The University of Hong Kong, Hong Kong**²Department of Radiology, The University of Hong Kong, Hong Kong*

Background: Lumbar disc displacement can lead to low back pain. It is believed that disc displacement is degenerative in origin, but controversy exists. Novel T1rho magnetic resonance imaging (MRI) is more sensitive than traditional T2-weighted MRI for assessing disc degeneration via quantifying loss of proteoglycan in disc. Hence, by utilising T1rho MRI, we addressed the 'proteoglycan profile' for each lumbar disc and level-specific threshold values as biomarkers for disc displacement.

Methods: This was a cross-sectional MRI study of 76 volunteers (380 discs; mean age 50.6 years and 51.3% male) who underwent T2-weighted and T1rho MRIs assessing the lumbar spine (L1-S1). Receiver operative characteristic curves were used to assess diagnostic T1rho values associated with disc displacement for each lumbar disc.

Results: Of all the discs, 50% had disc displacement, and the mean T1rho value for non-displaced discs was 77.6 ms compared with 64.5 ms for displaced discs. Optimal cutoff T1rho values for each lumbar disc level had been obtained as potential biomarkers for lumbar disc displacement.

Conclusion: This is the first study in humans to quantitatively assess 'proteoglycan profile' of disc displacement throughout the lumbar spine. Based on T1rho MRI, our group has identified a decrease in proteoglycan concentration in the presence of disc displacement at all levels. Level-specific values have been identified that may have predictive utility at the index or adjacent levels, which may aid in classification, aetiology, natural history, and therapeutics of disc displacement.