Free Paper Session VIII — Basic Science II

8.1

Manufacture of Human Engineered Tendon in an Ex-vivo Bioreactor System

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Introduction: Harvesting donor tendon and ligament tissue for tendon reconstructive surgery is problematic. This study aimed to examine feasibility of scaffold-free tendon engineered in a bioreactor using autologous tendon progenitor cells (TPCs) from a needle biopsy of tendon tissue.

Methods: Tendon progenitor cells were isolated from mice and from human patella tendon and characterised by colony-forming ability, flow cytometry, and differentiation assay. Cultured TPCs were treated with connective tissue growth factor and ascorbic acid before transfer to the bioreactor. Culture continued in either loading-free or mechanical stimulation (6% tensile strain at 0.25 Hz, 8h/day) conditions for 7 days. Histology, immunohistochemistry, qRT-PCR and mechanical test were performed to characterise the engineered tendon.

Results: We manufactured neotendon tissue that exhibited well-organised collagen structure with tenocyte cellular morphology. Immunohistochemistry showed the expression of type I collagen and tenomodulin in the tissue. Molecular assessment confirmed neotendon expression of tendon matrix molecules and specific transcription factors necessary for tendon maturation. Furthermore, mechanical stimulation further stimulated tenogenic differentiation of TPCs evidenced by increased expression of tenogenic markers and decreased expression of adipogenic, osteogenic, and chondrogenic markers at both gene level and protein level. Lastly, subjected to cyclic tensile loading, the neotendon showed comparable mechanical properties to the controls.

Conclusions: Autologous tendon cells from biopsy could generate neotendon tissue in bioreactor system. This is promising for tendon reconstruction applications.

8.2

The Relationship of Ligamentum Flavum Changes and Developmental Spinal Stenosis

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8.3

Biodegradable High-purity Magnesium Interference Screw Developed for Anterior Cruciate Ligament Reconstruction in Rabbit Model

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Introduction: How to accelerate the incorporation of the graft within the bone tunnel has been a concern for anterior cruciate ligament reconstruction (ACLR). Biodegradable magnesium (Mg), as the most promising osteopromotive medical device, may contribute to the improvement of tendon-bone attachment via consolidation of enthesis induced by Mg-involved microenvironment.

Materials and Methods: A total of 104 ACLR procedures were performed in rabbits with the use of long digitorum extensor tendon as the graft. Titanium or Mg screws were inserted into the femoral bone tunnels for fixation of the graft. Computed tomographic scanning was performed to monitor bone tunnel enlargement and trabecular bone remodelling at different regions of interest. Animals were sacrificed for mechanical, histological, and chemical tests after 3, 6, 12, and 16 weeks.

Results: In Mg group, early formation of mineralised Sharpey's fibres was present and convinced at 12 weeks via Stevenel's Blue staining, micro–Fourier transform infrared microspectroscopy, energy-dispersive X-ray analysis, and micro-hardness analysis. More formation of mineralised Sharpey's fibres was also detected for Mg group. Trabecular bones were reduced for both groups especially in the early stage. Although more serious bone tunnel widening was observed for Mg group, there were no significant differences in the maximum load between the 2 groups at different time points. According to the modified scoring system, no significant differences were recorded in healing quality of graft incorporation between both groups.

Discussion and Conclusion: Magnesium-based interference screws could accelerate the mineralisation of Sharpey's fibres for solidating the enthesis and may be considered for use in patients with anterior cruciate ligament rupture.

8.4

Low-dose Hydrogen Peroxide Impaired Tendon Healing and Induced Tendinopathic Changes

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Introduction: Oxidative stress has been implicated in the development of tendinopathy, but the direct effect of oxidative stress to impair tendon healing has not yet been investigated. In this study, the effect of imposed oxidative stress on tendon healing by local hydrogen peroxide administration was studied.

Materials and Methods: A patellar tendon (PT) window injury was created on the right knee in 24 male Sprague-Dawley rats. Three-weekly subcutaneous injections were given over the rat PT of either saline, 50 μM, or 500 μM H_2O_2 solution. Animal gait data and 3-dimensional power Doppler ultrasound imaging data were collected pre-injury and at 6 weeks postoperation. At day 42, the rats were euthanised for either histological (n=3) or mechanical (n=5) assessment.

Results: In the group with 50 μM H_2O_2 treatment, the elastic modulus of the injured PT was significantly lower compared with controls, with significant pain-associated gait asymmetry, hypoechogenic changes in ultrasound images, and increased power Doppler signals. Histological examination revealed significant degenerative changes and hypervascularity in the injured tendon treated with 50 μM H_2O_2.

Discussion and Conclusion: The results demonstrated that H_2O_2 impaired tendon healing and elicited tendinopathic changes, with respect to pain, structural abnormalities, and compromised mechanical strength. A higher dose of H_2O_2 did not elicit similar changes, which may be accounted by the triggering of host antioxidant defence mechanisms. The current findings suggested that oxidative stress may lead to failed tendon healing and could have a significant role in tendinopathies.

8.5

Subchondral Bone Cysts Indicate Osteopenia in Advanced Knee Osteoarthritis

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Introduction: Subchondral bone cyst (SBC) was reported as a predictor for cartilage loss and the risk of knee replacement, yet little is known about its consequence in osteoarthritis (OA) pathophysiology. We investigated the impacts of SBC on surrounding bone and marrow tissues via micro-computed tomography (μCT) and histomorphometry.

Methods: A total of 144 advanced OA knee replacement patients were selected. Trabecular parameters of resected tibial plateaux were analysed on μCT. Ratios of marrow tissue to total marrow space were calculated by histomorphometry.

Results: A total of 98 patients were SBC+ and 46 were SBC-. Presence of SBC in tibial spine was correlated to mean (± standard deviation) values of low mineral density (SBC+: 0.63 ± 0.047 g/cm^3 vs. SBC-: 0.65 ± 0.054 g/cm^3, p=0.011), bone volume fraction (17.62 ± 7.31% vs. 20.36 ± 8.44%, p=0.047), surface to tissue density (3.41 ± 0.78 mm^{-1} vs. 3.87 ± 0.91 mm^{-1}, p=0.004), trabecular number (0.92 ± 0.25 mm^{-1} vs. 1.07 ± 0.30 mm^{-1}, p=0.004), connectivity (7.16 ± 3.28 mm^{-3} vs. 8.55 ± 3.47 mm^{-3}, p=0.021), and increased trabecular separation (0.70 ± 0.10 mm vs. 0.64 ± 0.13 mm, p=0.004) in lateral compartment. The SBC+ samples also had higher tissue to marrow space ratio (21.9 ± 7.9%) than SBC- samples (11.1 ± 7.4%).

Conclusion: Subchondral bone cyst occurred together with low bone mass and marrow fibrosis. Subchondral bone cyst could be a biomarker for subchondral osteopenia in knee OA.

8.6

Bacterial 16S Ribosomal RNA Footprint Detected in Human Achilles Tendinopathy Samples

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Introduction: A number of chronic illnesses have been linked to microbes, and the immune response launched to tackle them. A number of these diseases have insidious structural changes similar to those in tendinopathy, for instance, intramuscular fibrosis in chronic myocarditis due to *Bartonella*. Furthermore, there have been cases of patellar and rotator cuff tendinopathy due to Lyme disease (*Borrelia*). Innate proinflammatory mechanisms in response to microbial components may lead to degradative changes in the tendon. To determine whether microbes can be implicated in tendinopathy, we sought to detect whether the bacterial 16S ribosomal RNA (16S rRNA) gene is present in human tendinopathy samples to support our hypothesis.

Materials and Methods: Our team gathered 13 Achilles tendinopathy samples (confirmed by magnetic resonance imaging) and 10 healthy hamstring and patellar tendon samples from anterior cruciate ligament reconstruction grafts under sterile conditions. Extracted DNA underwent polymerase chain reaction with universal 16S primers and imaged using gel electrophoresis. *Escherichia coli* was used as a positive control, while a blank reagent was used as a negative control.

Results: Five of these 13 Achilles tendinopathy samples and no healthy tendon samples were positive for 16S rRNA. There were significantly more tendinopathy samples with 16S rRNA than the healthy samples (Fisher's exact test, p=0.046).

Discussion and Conclusion: These results are the first of their kind to demonstrate the presence of bacterial footprints in tendinopathy samples. Further experiments are being conducted to determine the species of microbes present in the tendinopathy samples.

8.7

The Use of Thermo-sensitive Monomethoxypoly(ethylene glycol)-L-poly(alanine) Gel for Intra-articular Delivery of Quercetin to Treat Osteoarthritis

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Introduction: Quercetin is available as a nutraceutical for osteoarthritis (OA) patients. Due to its low bioavailability, a local administration may be preferable and we have demonstrated that intra-articular injection of quercetin resulted in short-term symptom relief in OA rats. To further improve the treatment efficacies, we proposed to develop a drug delivery system for sustained intra-articular delivery of quercetin.

Materials and Methods: Monomethoxypoly(ethylene glycol)-L-poly(alanine), which formed gel at body temperature, was used to encapsulate quercetin. In-vitro release of quercetin was investigated by incubating quercetin-loaded hydrogel in saline at 37°C for 30 days. Osteoarthritis was induced in 6 Sprague-Dawley rats by anterior cruciate ligament transection. At 6 months postoperation, an intra-articular injection of 50 μL of unloaded hydrogel or quercetin-loaded hydrogel (1 mg/mL) solution was given to rats (n=3). Gait analysis was performed before operation, at 3, 4, 5, 6 months postoperation, and at 1, 4, 8, and 12 weeks post-injection. At week 12 post-injection, the rats were sacrificed for histological scoring using the guideline recommended by the Osteoarthritis Research Society International histopathology initiative.

Results: Quercetin-loaded hydrogel showed a sustained release of quercetin up to 30 days with minimal gel degradation. Painful response as indicated by Limb Idleness Index decreased in the quercetin group at 4 weeks after injection, but not in the hydrogel-only group. Histological scoring showed no structural improvements in both groups.

Discussion and Conclusion: We demonstrated the safety and feasibility of the hydrogel for intra-articular drug delivery of quercetin to treat OA in rats.

8.8

Novel Chitosan Nanofibre Wound Dressing on Partial Thickness Skin Graft Donor Sites

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Introduction: Made of shells from shrimps and other sea crustaceans, chitosan is biodegradable, hypoallergenic, antibacterial, and haemostatic. Nanofibre technology enhances the surface-area-to-volume ratio, resulting in a very large contact area with the wound favouring cell growth and tissue regeneration. The Chitosan nanofibre dressing is a novel product developed by the Nanofibre research group (under the last author).

Methods: From 2012 to 2014, 10 patients were recruited from Kwong Wah Hospital as a pilot clinical study. Chitosan nanofibre dressing was applied to partial thickness skin graft (PTSG) donor site side-by-side to paraffin gauze dressing (by the first author). Wounds were assessed at 4 weeks, 3 months, and 6 months. Digital photos were assessed by a qualified plastic surgeon (the second author) at the conclusion of the study. Endpoints included any adverse events including infection, re-epithelisation time, and quality of scar assessed by the Vancouver Scar Scale (VSS) at 6 months.

Results: Chitosan demonstrated areas of protective scab at early period, which eventually fell off spontaneously by 2 to 3 months. Wound pain during removal of dressing was markedly reduced (visual analogue scale score, 7 vs. 2). Wound healed faster and had better scar quality, particularly in terms of pliability and pigmentation. The mean VSS score was 2.3/13. One patient suffered from donor site infection, after repeated skin harvesting twice, at both Chitosan and paraffin gauze–dressed area. This patient had VSS score of 7/13 for both arms. There was no other adverse event.

Conclusion: The novel Chitosan nanofibre wound dressing is a safe and effective dressing for PTSG donor sites.

8.9

Impaired Extracellular Matrix and Dendrite Formation in Primary Osteoblast Culture of Adolescent Idiopathic Scoliosis

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Introduction: Deranged bone morphometry and microarchitecture was found in adolescent idiopathic scoliosis (AIS) girls, implying abnormal bone turnover. Osteoblasts and their descendent osteocytes are key cells regulating bone quality via cell-to-cell communication and paracrine signalling. Our recent study revealed abnormal ultrastructure of osteocytes and lacuno-canalicular network (OLC) in AIS iliac crest bone biopsies, indicating dysfunctional transformation of osteoblasts to osteocytes in AIS which might affect bone structure. We hypothesised that the abnormal ultrastructure of OLC system was attributed to impair osteoblast transformation.

Methods: Primary osteoblasts were isolated from iliac crest bone biopsies collected from AIS subjects (n=10) and non-AIS controls (n=6) intra-operatively. Osteoblast transformation to osteocyte was induced by standard protocol. Cell metabolic activity was determined by Alamar Blue, and representative transformation markers expression and final outcome were determined by quantitative polymerase chain reaction, alkaline phosphatase, and Alizarin red staining (ARS).

Results: Alamar Blue results showed AIS and control osteoblasts with similar metabolic activity. Despite similar early differentiation stage indicated by expression trend of Runx2, AIS osteoblasts exhibited different transformation fashion with lower mineralisation (ARS), altered extracellular matrix markers (increased collagen I and reduced osteocalcin mRNA), and reduced dendrite formation marker (E11).

Discussion and Conclusion: This temporal study showed abnormal osteoblast transformation in AIS, suggesting impaired extracellular matrix and dendrite formation which could contribute to the observed abnormal ultrastructure of OLC system (unpublished data). Further study is merited to investigate the underlying mechanisms.

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8.10

Why Osteopenic Adolescent Idiopathic Scoliosis has Low Bone Mass and Deranged Bone Structure?

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Introduction: Deranged bone structure was found in about 30% of adolescent idiopathic scoliosis (AIS) girls with osteopenia. Given that osteopenia is a prognostic factor for curve progression, it is speculated that abnormal bone quality is associated to curve deformity. However, the underlying causes leading to osteopenia in AIS remain unclear. We hypothesised that bone mineralisation was abnormally low in osteopenic AIS.

Methods: In this case-control study, corticocancellous bone biopsies were collected from iliac crests of surgical AIS patients intra-operatively (17 osteopenic vs. 11 non-osteopenic girls) and subjected to structural and functional characterisation at tissue and cellular levels with micro-computed tomography, energy-dispersive X-ray spectroscopy, primary osteoblast and osteoclast cultures, respectively.

Results: Osteopenic AIS had lower calcium content than the non-osteopenic. The osteopenic AIS showed a general view of less bone volume fraction and trabecular number than the controls. Individual trabeculae segmentation further showed significantly less rod and plate volume and number, shorter rod length, and less connectivity in osteopenic AIS. Primary osteoblasts in osteopenic AIS showed obviously lower differentiation and calcium deposition. Osteopenic AIS showed higher osteoclastogenic potential. Conditioned medium from primary osteoblasts had higher pro-osteoclastogenic effect.

Discussion and Conclusion: This study for the first time provides detailed comparison between osteopenic and non-osteopenic AIS, and suggests that abnormal osteoblasts activities could contribute to the lower bone mass and deranged bone structure in osteopenic AIS.

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8.11

Abnormal Ultrastructure of the Osteocyte-lacuno-canalicular System in Adolescent Idiopathic Scoliosis — A New Novel Finding

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Introduction: Adolescent idiopathic scoliosis (AIS) is a complex 3-dimensional (3D) spinal deformity of unknown aetiology. Recently, our group reported abnormal proliferation and differentiation of osteoblasts from AIS to melatonin. Osteocytes, the descendent of osteoblasts, play important regulatory roles in bone homeostasis via interconnecting lacuno-canalicular network (collectively as OLC system). This study aimed to determine the ultrastructure of the OLC system from bone biopsies in AIS and age-matched controls.

Materials and Methods: A total of 5 iliac crest bone biopsies were taken intra-operatively from AIS patients and age-matched controls respectively under strict protocol approved by the institutional review board. The specimens were examined by acid-etched scanning electron microscopy (SEM) and confocal microscopy with quantitative fluorescein isothiocyanate–Imaris technique.

Results: In controls, SEM showed the well-organised osteocytes lacunae with typical spindle-like shape and the high connectivity of osteocytes with abundant perpendicular canaliculi protruding from the lacunae. While AIS osteocytes were more rounded and irregular in shape aligned in irregular clusters with shorter and disorganised canaliculi. Quantitative analysis of the 3D confocal images showed differences with a mean of 38% shorter, 44% less canaliculi, and 28% larger lacunar volume in AIS OLC system.

Discussion: To the best of our knowledge, this is the first study demonstrating the abnormal ultrastructure of OLC in AIS both qualitatively and quantitatively. Further studies will help to advance our understanding of the association between the abnormal bone quality and ultrastructure and its possible contributions to the aetiopathogenesis of AIS.

8.12

Ginsenoside Rb1 Attenuates the Progression of Arthritis in a Rat Osteoarthritis Model

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Osteoarthritis (OA) is the most common degenerated joint disorder. Inflammatory cytokine plays an important role in OA progression. Previous studies have demonstrated that ginsenosides Rb1 would prevent inflammation and apoptosis in chondrocytes. However, we do not find any animal study which reported the effect of Rb1 on attenuating the severity of osteoarthritis. In this study, we used a rat anterior cruciate ligament transaction plus medial meniscus resection (ACLT + MMx) model of OA to investigate whether continuously systemic administration of ginsenosides Rb1 might attenuate the progression of arthritis. The 16-week-old male Sprague-Dawley rats were divided into 3 groups: sham control group (group 1), Rb1-treated group (group 2), and OA group (group 3). For groups 2 and 3, OA was induced in the right knee joint with ACLT + MMx in rats. Then group 2 received continuous infusion of ginsenoside Rb1 via osmotic mini-pump implanted subcutaneously. At 4 weeks after treatment, the rats were sacrificed. Interleukin-1 (IL-1ß) level was evaluated by enzyme-linked immunosorbent assay; cartilage damage was assessed via histology (Safranin-O / fast green stain) and immunohistochemistry (MMP13, Col X). Characters of OA were present in the OA group, contrary to less severe damage in Rb1 treatment group in general: at first, IL-1ß level was significantly decreased; secondly, cartilage degeneration was attenuated, by lower histologic damage scores and the percentage of MMP13- or Col X–positive chondrocytes. In present study, we demonstrated that systemic administration of Rb1 can attenuate the progression or severity of arthritis via reducing inflammation level.

8.13

MicroRNA-146a Regulates Human Fetal Femur–derived Skeletal Stem Cell Differentiation by Down-regulating SMAD2 and SMAD3

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Introduction: MicroRNAs (miRs) play a role in a variety of biological processes including stem cell differentiation. Human fetal femur–derived skeletal stem cells (SSCs) display enhanced proliferation and multipotential capacity. This study aimed to examine the role of miRs in SSC function, particularly modulation of skeletogenesis.

Materials and Methods: Epiphyseal and diaphyseal cells were dissected from human fetal femur and extracted for monolayer culture. Expressions of osteogenic and chondrogenic marker genes were analysed using real-time quantitative polymerase chain reaction (RT-qPCR). The miR expression was profiled using a miR array. The miR-146a was overexpressed using lipofection and functional analysis was determined using Western blotting of Smad2 and Smad3 proteins. The effect of miR-146a overexpression on SSC differentiation was shown by examination of changes in gene expression demonstrated by RT-qPCR.

Results: Cells from the epiphyseal region of the fetal femur expressed higher levels of genes associated with chondrogenesis. Cells from the diaphyseal region expressed higher levels of genes associated with osteogenesis. The miR-146a was identified as an antichondrogenic miR and predicted to target various components of the transforming growth factor–beta pathway. The down-regulation of Smad2 and Smad3 following overexpression of miR-146a resulted in an upregulation of the osteogenesis-related gene RUNx2 and down-regulation of the chondrogenesis-related gene Sox9.

Discussion and Conclusion: The current findings indicate that miR-146a plays an important role in skeletogenesis through attenuation of Smad2 and Smad3 function and provides further insight into the role of miRs in human skeletal stem cell differentiation modulation with implications therein for bone reparation.