Conclusion: ER-α expression in osteoblasts was not enhanced by LMHFV, estrogen, and LMHFV+ICI treatments alone; but was enhanced when LMHFV and estrogen treatments were combined. LMHFV was previously shown to enhance ER expression and callus formation capacity detected in vivo [4]; the current observations suggest that the enhanced ER expression was not likely to come from local enhanced expression, but more likely due to systemic recruitment of ER-expressing reparative cells, such as mesenchymal stem cells [5]. LMHFV enhanced osteoblastic differentiation and osteogenic capacity, which was abolished by ICI 182, 780. ER is required in the signal transduction of LMHFV to callus formation capacity also supported by similar findings [6]. Therefore, LMHFV does not enhance ER-α gene expression at the cellular level in the absence of estrogen; and ER is required for the mechanical signal transduction leading to enhancement in osteogenic activities.

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Session: Traumatology — Fracture Healing

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IMPAIRED INFLAMMATORY RESPONSES IN OVARIECTOMY INDUCED OSTEOPOROTIC FRACTURE IN RAT MODEL

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Background: Mechanical stimulation has previously been reported to enhance fracture healing. We have reported mesenchymal stem cell recruitment, neoangiogenesis at the callus, endochondral ossification, and callus remodeling are impaired in osteoporotic fracture. Since these well-coordinated stages of fracture healing begin with the inflammatory stage, and all the subsequent stages are regulated by the release of inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) that have been reported to regulate MSC recruitment and angiogenesis. Therefore, it is hypothesized that the inflammatory response in osteoporotic fracture is impaired.
Methods: Ovariectomy-induced osteoporotic and sham-operated closed-femoral fracture SD-rat model (n=18, n=3 per group per time point) were used to investigate the differences in expression patterns of inflammatory factors in normal and OVX-induced osteoporotic fracture healing. SHAM and OVX were performed in 6-month-old SD rats and closed femoral fractures by Einhorn's protocol was performed at 9-months. Callus morphometry was determined by callus width from weekly radiography. Local expression of TNF-α and IL-6 was detected by immunohistochemistry and quantified by colour thresholding by ImageJ. IHC was assessed at week 2, 4, and 8 post-fracture. Significant difference between groups was considered at $p<0.05$ by the student's *t*-test.
Results: Callus width was detected to be lower at week 3, 4, and 5 compared to the SHAM group with statistical significance ($p<0.05$). Immunohistochemistry results generally showed that TNF-alpha and IL-6 were found to express at higher levels during the earlier phase of the healing process in the marrow space and also the periosteum lining cells during the healing process. Quantification results showed that there existed a difference in TNF-α and IL-6 expression at various time-points. Area fraction of TNF-alpha positive signal was found to be higher at 57±9% in SHAM rats versus 32±5% in OVX group at week 2 post-fracture ($p=0.012$). IL-6 signal was found to be higher at 40±6% in SHAM than 10±2% in OVX group at week 2 ($p=0.001$), 27±4% versus 7±1% at week 4 ($p=0.001$) and 18±3% versus 2.4±0.4% at week 8 ($p<0.0005$).
Conclusion: This pilot study has shown that the expression of the pro-inflammatory factors of TNF-α and IL-6 at the fracture callus exhibits a difference between normal and estrogen deficient osteoporotic fracture bone. Here we have shown that the expression of TNF-α impaired at week 2 in OVX, and IL-6 was consistently lower in OVX suggests that the inflammatory response in estrogen deficient rats had impaired inflammatory response after fracture injury that coordinate different fracture healing processes at different times including osteoblast and MSC differentiation. As ageing and sex hormone depletion during the development of osteoporosis both alter systemic inflammatory response[3], this supports that there exists a possible difference in the initiating stage of inflammation during osteoporotic fracture healing. Therefore, we report that there exists an impaired inflammatory response in OVX-induced osteoporotic fracture compared to normal fractures.

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Session: Clinical Studies — Outcome Assessment

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HIGH VOLUME HIP SURGEON ACTING AS AN ORTHOGERIATRICIAN DECREASES ONE-YEAR MORTALITY IN ELDERLY PATIENTS WITH FRAGILE HIP FRACTURE

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Hip fractures cause acute pain and loss of function, and often lead to hospitalization. After acute hip surgery, in-hospital mortality may be as high as 9.5% and one-year mortality as high as 14—36%. This study evaluated the risk factors related to one-year mortality in a medical center by analyzing the demographic, clinical, and surgical characteristics of the patients, as well as data on the medical provider. From January 2009 to December 2010, the records of 313 patients who received surgery for hip fracture were reviewed. Those with multiple fractures or combined trauma were excluded. Aside from descriptive statistics analyses were done using independent *t* test, analysis of variance, *Chi*-square test, linear regression, and logistic regression. The total complication rate was 53.7%. The most common complication was anemia. The one-year mortality rate was 12.1%, which was associated with co-morbidity, complication, and surgical care quality, particularly a specialized high volume hip surgeon acting as an orthogeriatrician. Despite the longer hospital stay and higher medical costs, the presence of a high volume surgeon was associated with a one-year mortality rate of 4.7% and a 67% reduction in mortality. One-year mortality was also related to more than three co-morbidities. Post-operative complications increased the one-year mortality. A high volume surgeon specializing in hip fracture patient care and acting as an orthogeriatrician can significantly reduce mortality. Nonetheless, combined care of these patients with geriatric specialists is strongly recommended, especially for those with more than three co-morbidities.

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Session: Biomaterials and Implants—Infection and Anti-Infective Implants

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HACC MODIFIED POLY (L-LACTIC-CO-GLYCOLIC ACID) ELECTROSPUN FIBROUS SCAFFOLD FOR GUIDED BONE REGENERATION MEMBRANE

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Background: Bone healing is one of the most important processes in clinical fields. Guided bone regeneration (GBR) is one such treatment that reconstructs neo-bone tissue by using a barrier membrane to prevent the ingrowth of fibrous tissues and guard subsequent osteoconduction. The popular membrane currently utilized for GBR is a non-degradable expanded polytetrafluoroethylene (e-PTFE), but it must be removed by a second surgery, which increases the risk of patient infection and other undesirable side effects. We seek to investigate the hydroxypropyltrimethyl ammonium chloride chitosan (HACC) surface modified poly(lactic-co-glycolic acid (PLGA) nanofibrous GTR membrane.
Subjects and Methods: The nanofibrous membranes were fabricated by the following electrospinning technique. HACC were covalently immobilized onto PLGA surface using N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS) in a 2-morpholinoethane sulfonic acid (MES) buffer system. The morphologies of the modified PLGA surface and the control were investigated by SEM. Elemental composition of the membranes surface was evaluated using an Energy Dispersive X-ray Spectrometer (EDX). The antimicrobial activity of the scaffolds was evaluated against *Staphylococcus aureus (*ATCC25923) and *S. epidermidis 287* (MRSE287). The proliferation of PDLCs on the substrates was examined with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 1, 4, and 7 days of culture. The cell morphology of the PDLCs was investigated by detecting the filamentous actin of the cytoskeleton using confocal laser scanning microscopy.
Discussion and Conclusion: The surface morphology of the HACC modified PLGA nanofiber was as smooth as that of untreated PLGA nanofibers. The results of water angle contact measurements test showed that the surface entrapment improved the hydrophilicity of the modified nanofibrous membranes. In addition, the

HACC modified electrospun PLGA nanofibrous membrane exhibited better biocompatibility and antibacterial properties than the PLGA nanofibrous membrane, which suggested the surface entrapment is a facile and efficient approach to surface modification for electrospun nanofibours membranes. Such a composite fiber membrane will provide a promising prospect for research and development in GBR membranes.
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Session: Disease & Treatment — Osteoarthritis

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ANTI-APOPTOTIC EFFECT OF mmu-miR-214-3p ON CHONDROCYTES FROM IL-1b-INDUCED APOPTOSIS

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Background: Osteoarthritis is a multifactorial degenerative disease that generally affected synovial joints. The apoptosis of chondrocytes play an important role in the pathogenesis of OA. Here we study the effect of mmu-miR-214-3p on the apoptosis and proliferation of mouse chondrocytes.
Subjects and Methods: Chondrocytes of C57/Bl6 mice were exacted and cultured in vitro. Chondrocytes were then transfected with mimics or inhibitor of mmu-miR-214-3p with or without IL-1β (10ng/ml) treatment for 24 to 48 hours. The proliferation and cell viability were evaluated by CCK-8 test. The apoptosis rate was detected by Annexin-V/7-AAD flow cytometry and tunnel staining. A quantitative colorimetric assay was used to determine activated caspase-3. mRNA expression of Bim, Bcl-2, Bad, AIF, PTEN were evaluated by real-time PCR, and the total Akt, phosphorylated Akt (p-Akt), Bcl-2, Bad, cytochrome C (Cyt c) were determined by Western blotting.
Results: There were no significant differences in absorbance between the miR-214-3p mimics group (50nM, 100nM, 200nM), miR-214-3p inhibitor group (100nM, 200nM), mimics NC and inhibitor NC group for cell proliferation. Cell viability was significantly reduced in IL-1β treated group. However the over expression of mmu-miR-214-3p (50nM, 100nM) decreased cell damage caused by IL-1β ($P<0.05$).There were no significant differences in apoptotic rate between over expression or knock down of mmu-miR-214-3p and NC. Over expression of mmu-miR-214-3p (50nM) can significantly reduce the apoptotic rate of chondrocytes induced by IL-1β ($P=0.003$).Expression of activated caspase-3 was lower for mmu-miR-214-3p mimics+ IL-1β group than IL-1β group and mimics NC+ IL-1βgroup. Mmu-miR-214-3p mimics transfection could reduce the mRNA expression of Bim, Bcl-2 and PTEN ($P<0.05$, $P<0.05$and $P<0.01$), whereas mmu-miR-214-3p knock down could improve the mRNA expression of Bim, AIF and PTEN ($P<0.01$, $P<0.05$and $P<0.05$). mmu-miR-214-3p mimics transfection improved p-Akt and Bcl-2 protein expression ($P<0.05$, $P<0.05$) and reduced Bad, Cyt c expression($P<0.01$, $P<0.05$)
Discussion and Conclusion: The over expression or knock down of mmu-miR-214-3p alone did not exhibit a significant influence on the proliferation and apoptosis of chondrocytes. However, over expression of mmu-miR-214-3p showed an anti-apoptotic effect on IL-1β induced apoptosis, which indicated that mmu-miR-214-3p may be related to the inflammatory course in OA. The protected effect of mmu-miR-214-3p against apoptosis probably via PI3K/Akt pathway.
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Session: Regenerative Medicine — Growth Factors

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FIBROBLAST GROWTH FACTOR-2 IMPROVES THE TENDON-TO-BONE HEALING BY STIMULATING THE GROWTH OF TENOGENIC PROGENITORS IN A RAT ROTATOR CUFF REPAIR MODEL

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Background: Certain growth factors are noted to enhance healing after rotator cuff (RC) surgical repair. Fibroblast growth factor (FGF)-2 has the potential to promote tendon-to-bone healing after RC repair in rats; however, this mechanism remains unclear. FGF-2 stimulates self-renewal of mesenchymal stem- or progenitor cells; thus, we hypothesized that FGF-2 administration to reparative sites may promote tenogenic-progenitor growth, resulting in biomechanical and histological improvement of repaired rat RCs. This was tested in a rat RC repair model by analyzing early, scleraxis (Scx)—a basic helix-loop-helix-family transcription factor—and late, tenomodulin (Tnmd)—a type II transmembrane protein—tenogenic marker expression. Histological evaluation and biomechanical testing were also performed.
Methods: Adult male Sprague—Dawley rats ($N=156$) underwent unilateral surgery to reattach the supraspinatus tendon to its insertion site. We used biodegradable gelatin hydrogel sheets as carriers for FGF-2, placed between the supraspinatus tendon and bone. Two groups were assessed for the effects of FGF-2 on RC healing: an FGF-2 (5 μg)—treated group and a control group (carrier only). At 2, 4, 6, 8, and 12 weeks post-operation, tenogenic-marker (Scx and Tnmd) expressions in reparative tissues were evaluated using real-time reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization (ISH). Healing sites were evaluated using semi-quantitative histology. At 12 weeks, biomechanical testing was performed. Statistical significance was estimated using the Mann-Whitney *U*-test; $p < 0.05$ was considered statistically significant.
Results: Real-time RT-PCR analysis demonstrated that Scx and Tnmd expression levels increased significantly in the FGF-2—treated group from 4—8 and 4—12 weeks postoperatively, respectively. ISH analyses showed that Scx and Tnmd transcripts were mainly observed in the spindle-shaped cells of the tendon mid-substance in both groups, and these signals tended to expand into reparative tissues to a greater extent in the FGF-2—treated than in the control group at ≥6 weeks. The FGF-2—treated group demonstrated a tendon-like tissue with oriented collagen fibers between tendon and bone compared to the control group; consequently, histological scores were significantly higher in the FGF-2—treated than in the control group at ≥4 weeks. At 12 weeks, biomechanical testing showed that the ultimate load-to-failure (ultimate load) and ultimate stress-to-failure (ultimate stress) were significantly higher in the FGF-2—treated group than in the controls (ultimate load: 23.8 ± 4.6 N vs. 13.8 ± 4.7 N; ultimate stress: 6.9 ± 1.1 N/mm^2 vs. 3.9 ± 1.1 N/mm^2). No differences were observed in the stiffness and cross-sectional area of the repair site.
Discussion and Conclusion: In the FGF-2-treated group, expression of Scx and Tnmd increased at ≥4 weeks, indicating that more tenogenic progenitors were included in the reparative site. Improvement of histological parameters, such as fibrovascular tissue reduction and collagen orientation at the insertion site, was observed at ≥4 weeks. Biomechanical strength also increased at 12 weeks. Collectively, this suggests that FGF-2 promotes growth of tenogenic progenitors at the reparative site, which contribute to tendon-to-bone healing. These findings provide insight into the clinical development of biological enhancement strategies for RC healing.
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Session: Disease & Treatment — Pharmaceutical Interventions

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LOCAL INTRA-ARTICULAR INJECTION OF RESVERATROL DELAYS ARTICULAR CARTILAGE DEGENERATION BY REGULATING HIF AND THEREBY PROMOTING CHONDROCYTE AUTOPHAGY

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Objective: Autophagy is an essential cellular homeostasis mechanism that was found to be compromised in aging and osteoarthritis (OA) cartilage. Previous studies showed that resveratrol can effectively regulate autophagy in other cells. The purpose of this study was to determine whether the chondroprotective effect of resveratrol was related to chondrocyte autophagy and to elucidate underlying mechanisms.
Methods: OA model was induced by destabilization of the medial meniscus (DMM) in on 10-week-old male mice. OA mice were treated with resveratrol with or without 3-MA for 8 weeks beginning 4 weeks after surgery. The changes of articular cartilage structure were examined by using semiquantitative scoring systems and Safranin O-fast green. The protein expressions of ULK1, LC3, Beclin1, HIF-1α, HIF-2α, AMPK, p-AMPK, mTOR, p-mTOR, COL2A1, Aggrecan, MMP13, ADAMTS5 expressions were analyzed by Western blot. COL2A1, Aggrecan, MMP13, ADAMTS5 mRNA expressions were analyzed by Real-time PCR.
Results: The local intra-articular injection of resveratrol delayed articular cartilage degradation in DMM-induced OA. Resveratrol treatment increased LC3, ULK1, Beclin1, HIF-1α, p-AMPK, COL2A1, Aggrecan expressions, but decreased HIF-2α, p-mTOR, MMP13and ADAMTS5 expressions. The effects of resveratrol were obviously blunted by 3-MA except HIF and AMPK.
Conclusion: The study demonstrated that resveratrol intra-articular injection delayed artilage degeneration and promoted chondrocyte autophagy in an experimental model of surgical DMM-induced OA, in part via activating AMPK signaling