IBDW2014-00133-F0056
QUANTIFICATION OF CARTILAGINOUS CALLUS BY CONTRAST ENHANCED MICRO COMPUTED TOMOGRAPHY IMAGING FOR FRACTURE HEALING EVALUATION
Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong

Objective: Formation of soft cartilaginous callus to stabilize the fracture site is an important stage in the healing process. Computed Tomography (CT), which is a non-destructive approach, can be a useful tool in the studies of fracture healing. However, the low radio-opacity of cartilage hampered the use of CT as it is nearly invisible on radiographs or CT scans in the early stages of healing.

Methods: This study used a combination of a commercially available anionic contrast agent known as Hexabrix® and micro-computed tomography (μCT) to visualize the soft cartilaginous callus around the fracture site in rats. A transverse, unilateral and closed fracture was made on the right femur of each rat. Fracture calluses were scanned before and after the incubation in different concentrations of Hexabrix® from 10% to 40%, resulting in non-uniform distribution of anionic contrast agent depending on the electro-chemical interaction between the contrast agent and negatively-charged sulfated glycosaminoglycans (sGAGs) in the cartilage. The CT images were then segmented, reconstructed and evaluated for both morphological and compositional analyses. The volumes identified with contrast-enhanced computed tomography (CECT) were compared with the area obtained from the gold standard histological assessment.

Results: Dilution of anionic contrast agent to 20% was found to be the optimal concentration that allowed both qualitative identification and quantitative analyses of the cartilages. The callus volume measured using 20% contrast agent with CECT was also strongly predictive of the corresponding histological measurements.

Conclusion: These findings show that non-invasive CECT allows visualization of soft cartilaginous callus and quantification of the callus composition, enabling a more comprehensive examination of fracture healing on animal models thanks to the non-destructive feature that allows future mechanical testing.

IBDW2014-00134-F0057
RESTORATION OF ESTROGEN RECEPTOR EXPRESSION IN OSTEOPOROTIC FRACTURE BY LOW MAGNITUDE HIGH FREQUENCY VIBRATION THERAPY
Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong

Introduction: Osteoporotic fracture healing was shown to respond differently to mechanical stimulation from normal fracture, possibly due to delayed estrogen receptors (ER) expression for mechanical signal transduction. We hypothesized that Low-Magnitude High-Frequency Vibration (LMHFV) could restore ER-α and ER-β gene expression, thus accelerate osteoporotic fracture healing.

Methods: Seventy-two osteoporotic SD rat fracture model was used to investigate the effect of LMHFV on the callus morphometry, estrogen receptor expression quantified by real-time PCR, and ER-immunohistochemistry (IHC) and micro-CT. Fractured rats were assigned to SHAM, OVX and OVX-VT group, assessed at 2, 4, 8 weeks post-fracture (gene: n=5/group/time-point, IHC: n=3/group/time-point).

Results: SHAM and OVX-VT showed higher callus size than OVX group from 2 to 4 weeks (all p<0.05). Both ER-α (p=0.001) and ER-β (p=0.003) gene expression was higher in the CTRL and OVX-VT groups at 2 weeks. BMP-2 expression was higher at week-2 (p=0.007) and week-4 (p=0.001) in SHAM and OVX-VT groups. IHC showed generally stronger signal in the SHAM and OVX-VT groups than the OVX group at week 2. Micro-CT showed higher low-density callus volume in the OVX-VT group.

Conclusion: Osteoporotic fracture healing was shown to be accelerated by LMHFV by callus morphometry. ER and BMP-2 expression levels were shown to be comparable between SHAM in OVX-VT at early stage of fracture healing over the OVX group. IHC also demonstrated similar observation. We conclude that expression in ERs in osteoporotic fracture healing could be restored by LMHFV, thus leading to differential healing responses to mechanical stimulation.

Acknowledgements
This study was supported by the AADO Research Fund (AADO-RF2010-001-2Y); and the CUHK Direct Grant for Research (2009.1.044).
IBDW2014-00136-F0059
COMPARATIVE STUDY OF TWO TYPES OF BIOACTIVE EPIMEDIUM FOR OSTEOPOROSIS TREATMENT IN AN OVARIECTOMIZED RAT MODEL
Shi Hui Chen a, Xin Luan Wang a,b, Yang Bai a, Li Zhen Zheng a, Ling Qin a,b
aDepartment of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, China
bTranslational Medicine R&D Center, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

Background: Osteoporosis is a systemic metabolic disease causing a reduction of bone mass and strength, and an increase in fracture risk consequently. Traditional Chinese Herbs, e.g. Epimedium are often used as a major composition to formulate herbal formula for osteoporosis treatment. Different locality of herbs may cause different properties that would be a decision of herbal formula selection for R&D of anti-osteoporosis herbal drugs. This study aims to compare the efficacy of two Epimedium species, namely Epimedium koreanum Nakai (X1) and Epimedium pubescens Maxim (X2).

Methods: Ovariectomized (OVX) model was established in sixty female Sprague Dawley rats (220±20g). High (90 mg/kg/day) and low (45 mg/kg/day) dosages of X1 and X2 were designed for daily intragastric administration for 90 days post OVX, namely X1L, X1H, X2L and X2H groups, also sham-operated rats (sham group) and OVX rats (OVX group). After scarifying the animals, micro-CT scanning of the proximal tibia and the fourth lumbar vertebra was conducted for evaluations of bone mineral density (BMD), bone tissue volume fraction (BV/TV) and trabecular number (Tb.N). Biomechanical properties of the mid-shaft of tibia and the fourth lumbar vertebra were also obtained.

Results: Micro-CT results of proximal tibia showed that as compared to OVX group, significant higher BMD (28.41% and 25.35%) and BV/TV (33.01% and 38.33%) were found in X1H and X2L groups, respectively (p<0.05, n=6). For micro-CT results of vertebrae, significant higher BMD (23.72% and 22.90%), BV/TV (31.70% and 30.96%) and Tb.N (26.85% and 18.27%) were presented in X1H and X2L groups, respectively (p<0.05, n=6). Based on the results of 3-point bending test in tibia, there was no significant difference of the mechanical strength between OVX group and each Epimedium groups (p>0.05, n=6), however, the results of compression test indicated the significant higher E-Modulus (81.82%, 80.21% and 72.04%) and compressive strength (74.59%, 64.92% and 81.05%) in X1L, X1H and X2L groups, respectively (p<0.05, n=6).

Conclusion: The two species of Epimedium showed significant efficacy for slowing the loss of bone mass and strength in osteoporosis, which presented a potential dose effect for both of the two species. However, there was no significant difference found between the high dose of X1 and low dose of X2 which indicated that both two Epimedium species could be selected for R&D of herbal drug.

Acknowledgement
The study was supported by 12.5 Major New Drug Creating Special Projects from the Ministry of Sciences and Technology of China (Ref. No: 2011ZX09201-201-05).