(Dcomp), and the cortical thickness (Ct.Th,mm). New features, such as cortical porosity (Ct.Po,%), pore volume (Ct.PoV,mm3) and mean pore diameter (Ct.Po.Dm,mm^2) were measured by an auto-contouring process. The cortical thickness derived from the auto-contour (Ct.ThautoC) was also obtained. All tibia were harvested in four quadrants at the same position of HR-pQCT measurements (9 mm height) for the conventional micro-CT analyses performed with a Skyscan 1172 ® device (voxel size=7.5μm). The posterior quadrant was also imaged by synchrotron radiation (SR) micro-CT at the ESRF Beam line ID 19 (voxel size=7.5μm).

First, site matched analyzes were performed to compare SR with conventional X-rays micro-CT results. Pore volume, (PoV), porosity (PoV/TV), pore size (Po.Si), pore spacing (Po.Sp), pore number (Po.N) and the degree of anisotropy (DA) were measured in site matched areas with micro-CT comparatively to HR-pQCT images. The cortical thickness (Ct.Thmicro-CT) was manually measured. Secondly, from conventional micro-CT images, the parameters of the cortical bone were averaged from the 4 quadrants and were compared to those from HR-pQCT images.

Results: The correlation coefficients between parameters from SR and conventional micro-CT were ($r=0.95$, $p<10\text{-}4$) for PoV, ($r=0.98$, $p<10\text{-}4$) for Po/TV, ($r=0.86$, $p<10\text{-}4$) for Po.Sp, ($r=0.76$, $p<10\text{-}4$) for CtThmicro-CT, ($\rho=0.71$, $p<0.001$) for Po.Si, and the coefficients were not significant for Po.N and DA.

The correlation coefficients of Ct.Thmicro-CT versus Ct.Th or Ct.ThautoC were high: $r=0.88$ $p<0.001$ and $r=0.84$, $p<0.001$, respectively. Dcomp were highly correlated to PoV/TV ($r=-0.83$, $p<10\text{-}4$). The Ct.Po versus PoV/TV($r=0.62$, $p<0.04$), Ct.PoDm versus Po.Si were not correlated ($r=0.47$, $p=0.14$), and CtPoV ($r=0.54$, $p<0.08$) was marginally correlated to PoV.

Conclusion: Distal tibia is a reliable region to study cortical bone with HR-pQCT measurements with Dcomp as the best parameter because it reflects both the micro-porosity (Havers canals) and macro-porosity (resorption lacunae) of the cortical bone.

IBDW2014-00082-F0014
VEGF-SRC SIGNALING IS ESSENTIAL FOR VASCULAR ENDOTHELIAL PERMEABILITY AND OSTEOCLASTS ACTIVITY

Huijuan Cao [a,b], Lizhen Zhengb [a,b], Nan Wang [a,b], Dan Li [a,b], Linying Wang [a,b], Xinluan Wang [a,b], Ling Qin [a,b]
[a]*Translational Medicine R&D Center, Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, PR China*
[b]*Musculoskeletal Research Laboratory, Department of Orthopaedics& Traumatology, The Chinese University of Hong Kong*

Objectives: To delineate the role of VEGF and c-Src signals in triggering destructive repair of osteonecrosis in vitro.

Materials and methods: The primary endothelial cells and osteoclasts were adopted in this study. Pharmacological VEGF and Src specific pp60c-srcsiRNA were used to determine the contribution of VEGF-Src signaling to vascular permeability and osteoclasts activity. Cells were treated with 50 ng/ml VEGF and/or transfected with the pp60c-srcsiRNA every other day. In parallel, equivalent PBS and non-targeting siRNA were treated in the control groups. We analyzed the endothelial permeability associated structural elements and the osteoclast formation and function.

Results: Results showed that the appropriate pp60c-srcsiRNA significantly reduced Src expression both in the endothelial cells and osteoclasts. For decreasing VEGF-mediating higher vascular permeability, Src blockade significantly relieved actin stress and the formation of caveolae and vesiculo-vacuolar organelles (VVOs), as well as stabilized the complex beta-catenin/VE-cadherin/Flk-1 through decreasing phosphorylation of VE-cadherin, to keep endothelial junction integrity. In addition, VEGF promoted osteoclasts formation and function, while the adhesion activity and cytoskeleton were not obviously affected by VEGF. However, Src blockade significantly destroyed the cytoskeleton resulting in a lower adhesion activity and inhibited the osteoclasts differentiation and function through decreasing the phosphorylation of Src, Pyk2 and Cbl. These findings indicated that Src blockade not only reduced the VEGF mediating vascular permeability, but also reduced osteoclasts activity.

Conclusion: VEGF-Src signaling is essential for vascular endothelial permeability and osteoclasts activity. Thus, blockade of VEGF-Src signaling may provide us a new view to develop novel strategies for preventing and treatment of destructive repair in osteonecrosis.

Acknowledgements
This work was supported by NSFC (81171771) and HKGRF (473011).

IBDW2014-00083-F0015
EFFECT OF PSORALIDIN ON INHIBITING ADIPOGENESIS—AN *IN VITRO* EFFICACY AND MECHANISTIC STUDY

Nan Wang [a,b], Xin-Luan Wang [a,b], Hui-Juan Cao [a,b], LinYing Wang [a,c], Ling Qin [a,d]
[a]*Translational Medicine R&D Center, Shenzhen Institutes of Advanced Technology Shenzhen, 518055, China*
[b]*Shenzhen Bioactive Materials Engineering Lab for Medicine, Shenzhen, PR China*
[c]*Nano Science and technology Institute, The University of Science and Technology of China*
[d]*Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong SAR*

Objective: Psoralidin, an coumarins extracted from the seed of Psoralea corylifolia L., and found it has estrogen-like activity that is mediated through estrogen receptors. In this study, we aimed to investigate the effects and molecular mechanism of psoralidin on adipogenesis dependent of ER signaling in vitro.

Methods: The cytotoxicity of psoralidin on 3T3-L1 preadipocytes and MCF-7 cell line was investigated by CCK-8 kit. Oil Red O staining in 3T3-L1 cells were used to demonstrate the effects of psoralidin on adipogenesis. The real time PCR was used to detect the mRNA expressions of the adipocyte-related genes, such as CCAAT/enhancer binding protein α (Cebpα), peroxisome proliferator-activated receptor γ (Pparγ), adipocyte lipid-binding protein (Fabp4) and lipoprotein lipase (Lpl). In addition, the protein expression of PPAR-γ, C/EBP α, Fabp4, LPL, phosph-GSK-3β-Ser9 and phosph-AKT-Ser473 were detected by western blot assay. All quantitative data were presented as means ± SD of three experiments.

Results: Psoralidin had no cytotoxicity effect on 3T3-L1 cell line, but it could significantly promote MCF-7 cells proliferation on selected dosage at 48 hours treatment. Psoralidin decreased the adipocytes in a dose dependent manner, as well as down-regulated the mRNA and protein levels of Cebpa, Pparg, Fabp4 and Lpl, but these effect would be weaken, even disappeared when co-treated with ICI182,780. The protein expression of phosph-GSK-3β-Ser9 and phosph-AKT-Ser473 on 3T3-L1 should be further proceed. These results suggested that psoralidin could inhibit adipogenesis, which might be through ER signaling pathway.

Conclusions: Psoralidin can inhibit adipogenesis in vitro. The underlying mechanism might be through ER signaling pathway.

Acknowlegement
This project was granted by Eu-NSFC grant (51361130034, FP7-604517), NSFC grant (81302782) and HKGRF grant (d00574).

IBDW2014-00084-F0016
A NOVEL MAGNESIUM COMPOSED PLGA/TCP POROUS SCAFFOLD FABRICATED BY 3D PRINTING FOR BONE REGENERATION

Yuxiao Lai [a], Long Li [a], Shukui Chen [a], Ming Zhang [a], Xinluan Wang [a], Peng Zhang [a], Ling Qin [a,b]
[a]*Center for Translational Medicine Research and Development, Shenzhen Institute of Advanced Technology, Chinese Academy of Science, China*
[b]*Musculoskeletal Research Laboratory, Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong*

Introduction: Bone regeneration is a crucial event in bone tissue engineering, and bioactive scaffold has become a focused strategy. Magnesium is a biodegradable and bioactive metal with needed mechanical strength for bone healing. An innovative Mg associated bioactive porous scaffold composed of poly (lactide-co-glycolide, PLGA), β-tricalcium phosphate (TCP) and magnesium (Mg) with well-defined biomimic microstructure for bone regeneration was designed and fabricated by low-temperature 3D printing technology. This PLGA/TCP/Mg scaffold has good biocompatibility and needed mechanical strength close to human trabecular bone and suitable for bone reconstruction. This study presented the enhancement of magnesium in mechanical properties and biocompatibility of the composite scaffold. The structure and mechanical properties and in vitro biocompatibility of this scaffold were investigated.

Results: The PLGA/TCP/Mg scaffold fabricated by low-temperature rapid-prototyping(LT-RP) with well-defined structure had high porosity with regular macropores (around 450 μm) and numerous micropores ranging from 2.5 μm to 90 μm distributed on the pore wall of the scaffold (see Fig.1). The high-resolution micro-computed tomography (micro-CT) results showed that the scaffold porosity was above 85% and the connectivity was almost 100%. The mechanical strength of the PLGA/TCP/Mg scaffolds was enhanced