(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.

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

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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.

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EFFECT OF PSORALIDIN ON INHIBITING ADIPOGENESIS—AN *IN VITRO* EFFICACY AND MECHANISTIC STUDY

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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.

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

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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

with increasing Mg content. The Young's modulus of PLGA/TCP/Mg scaffolds (Mg content: 15% wt) was around 104 Mpa, statistically significantly stronger than that of Mg content: 10% wt (83Mpa) and Mg content 5% wt group (82 Mpa), as well as PLGA/Mg group, 66Mpa. All the Mg containing scaffolds were statistically significantly stronger than PLGA/TCP and PLGA group, 45 Mpa and 30 Mpa, respectively.
Conclusion: The results of CCK-8 assay demonstrated the MC3T3-E1 osteoblasts grew very well and proliferated rapidly on PLGA/TCP/Mg scaffolds compared to PLGA/TCP scaffold after 7 day culture. The in vitro study also demonstrated a good biocompatibility and bioactivity of the PLGA/TCP/Mg scaffold that was in favor of accelerating and inducing the proliferation and differentiation of osteoblasts.
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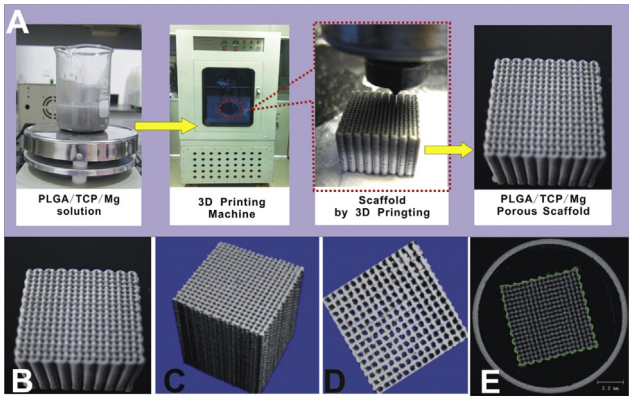

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SYNERGISTIC EFFECT OF DECELLULARIZED ANNULUS FIBROSUS (AF) MATRIX AND SUBSTRATE STIFFNESS ON THE GENE EXPRESSION OF RABBIT AF STEM CELLS

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Objective: Decellularized matrices (DCM) have been widely used for engineering functional tissues, mainly due to the similarity of biochemical composition and microstructure between them and native extracellular matrix (ECM) in vivo. Meanwhile, the mechanical properties of substrate play an important role in regulating cell behavior such as adhesion, proliferation, differentiation and migration. Here, we aimed to study the combined effect of DCM and substrate stiffness on the behaviors of newly identified rabbit AF-derived stem cells (AFSCs).
Methods: To this end, decellularized porcine annulus fibrosus matrix (DAFM) was covalently coupled to polyacrylamide gels (PAGs) which had elastic moduli of 2.6 KPa (Soft), 10.6 KPa (Middle), and 34.9 KPa (Rigid), respectively. As control, collagen-coated PAGs of similar stiffness were used.
Results: After 7 days of culture on these PAGs, AFSCs on soft collagen-coated PAGs exhibited the least expression of collagen-I gene, while cells on rigid collagen-coated PAGs exhibited the greatest. In contrast, cells on soft collagen-coated PAGs exhibited the greatest expression of collagen-II and aggrecan genes, while cells on rigid collagen-coated PAGs had the least expression of them.
Conclusion: The gene expression of cells on middle PAGs was between those on soft and rigid PAGs. Expression of the above genes in AFSCs cultured on DAFM-coated PAGs followed similar substrate stiffness-dependent pattern. However, the responses of AFSCs to substrate stiffness appeared to be more prominent when they were cultured on DAFM-coated PAGs. Therefore, combined use of DAFM and scaffolds of gradient stiffness may provide a more efficient approach for AF tissue engineering.

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ABNORMAL FUNCTIONAL RESPONSES OF OSTEOBLASTS TO LEPTIN IN ADOLESCENT IDIOPATHIC SCOLIOSIS

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Objective: Leptin has been postulated as one of the etiologic factors of AIS because of its important physiological functions in neuro-osseous development affecting skeletal growth, the onset of puberty, energy expenditure and body composition. Previous studies on the relationship between leptin and HR-pQCT derived bone quality parameters had found abnormal correlations in AIS girls, and suggested possible abnormalities in the leptin regulated bone metabolic pathways. Another study on AIS patients showed hyposensitivity to leptin in bone marrow derived mesenchymal stem cells. This study aimed to investigate the effect of leptin on the functional responses of osteoblasts in AIS girls, and compare with that in control subjects.
Material and Methods: In vitro assays were performed with osteoblasts isolated from 12 severe AIS girls and 6 control subjects. The osteoblasts were exposed to different concentrations of leptin (0, 10, 100, 1000 ng/ml). The effects of leptin on cell proliferation were evaluated with MTT assay after 3 days of leptin treatment; differentiation with ALP activity assay after 6 and 14 days, and with osteocalcin ELISA throughout the 35 days of culture; and mineralization with von Kossa staining after 21 and 35 days.
Results: Baseline comparison between osteoblasts from AIS and control groups showed lower differentiation and mineralization potentials in the AIS group. For functional responses to leptin, control group showed increasing proliferative response to leptin in a dose dependent manner ($p=0.008$), while AIS group showed no proliferate response to leptin ($p=0.962$). For differentiation, control group showed strong and significant trend in ALP activity to increasing leptin concentrations in both day 6 ($p=0.012$) and 14 ($p=0.017$), and secreted osteocalcin in an increasing dose dependent manner to leptin ($p=0.007$ in day 35), but this trend were not observed in the AIS group ($p>0.05$). For mineralization, the control group showed a mild rising trend to increasing leptin concentrations ($p=002$), and again no trend was observed in the AIS group ($p=0.305$).
Conclusion: The results in this study suggested that the osteoblasts isolated from AIS girls had low differentiation and mineralization potentials, as well as abnormally low functional responses to leptin when compared with controls. These decreases in functional responses might be due to dysfunction of leptin signaling pathway, which could include abnormalities in the leptin receptor or downstream signal molecules. This is an important finding and might serve to explain the low bone mass and deranged bone quality that is associated with AIS.

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BONY SPUR FORMATION AND DISCUSSION IN COLLAGEN-INDUCED ARTHRITIS RAT MODEL

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Introduction: A systemic chronic joint inflammation leads to profound changes in the joint architecture, which is the structural basis for progressive impairment of function. Radiographic features of RA are those of joint inflammation, periarticular osteopenia, uniform joint space loss, bone erosions, and soft-tissue swelling. Conversely, inflammatory joint destruction is sometimes accompanied by modeling of bony spurs, also termed osteophytes, which emerge at the joint margins in diseases, such as psoriatic arthritis and ankylosing spondylitis. The reason for the apparently divergent bone responses among various inflammatory diseases has not been fully clarified, but appears to involve differential regulation of local bone homeostasis in the course of joint inflammation. With the destruction of joint