drive MEFs to an intermediate stage. Then chondrogenic growth factors were used to complete the induction by 2D/3D culture. Cells of each stage were characterized by immunofluorenscence and single cell sequencing. A col2-pd2EGFP reporter system played a role in the following high-throughput screening for efficiency optimization.
Results: Col2+ chondrocyte-like clusters could be visualized after chemical induction by 2D/3D culture. Consequently, 3D culture (micromass and suspension culture) with TFGβ3 promoted the chondrogenesis of chemical-induced MEFs compared to monolayer culture, resulting in a cartilage-like particle. For the whole chemical lineage conversion, the first stage was essential, during which fibroblasts were driven into an intermediate stage with pluripotent colonies and heterogenous cell subpopulation. With the help of col2-pd2EGFP screening system, we discovered that adding other 2 small molecule compounds during the first stage significantly increased the fibroblast-to-chondrocyte conversion efficiency.
Conclusion: This study demonstrates the possibility of chemical conversion from mouse fibroblasts to cartilage-like tissue without genetic manipulation. This proof of concept study lays a foundation for high-throughput screening of chondrogenic inducers and *in vivo* chemical conversion in clinical cartilage repair.
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Session: Disease & Treatment — Osteoarthritis

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DIFFERENT EFFECTS OF PREDNISONE TREATMENT FOR 30 DAYS AND 90 DAYS ON BONE METABOLISM IN COLLAGEN-INDUCED ARTHRITIS (CIA) RATS

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Background: Glucocorticoids (GCs) are often prescribed to treat RA for a long time, but there is still controversy in the administration of GCs, mainly because of the adverse reactions, such as osteoporosis. How to control the dosage and duration of GCs treatment is the problem we need to solve. The aim of this study was to investigate the different effects of prednisone treatment for 30 days and 90 days on bone metabolism in collagen-induced arthritis (CIA) rats and hope to provide a reasonably clinical application of glucocorticoids for the treatment of RA.
Subjects and Methods: Fifty 8-week-old female Lewis rats were randomly divided into 2 groups, normal group with 12 rats and the remaining 38 rats were used to establish the CIA model. Four weeks after immunization, 24 rats which were successfully infected with arthritis (arthritis index $\geq$4) were randomly divided into CIA treated with vehicle group and CIA treated with prednisone group. 6 rats were killed in each group on the 30d and 90d respectively. Bone histomorphometry, bone mineral density (BMD), micro-computer tomography (micro-CT), biomechanical test and enzyme-linked immunosorbent assay were performed.
Results: Compared with normal rats, in fourth lumbar vertebrae (LV4), percent trabecular area (%Tb.Ar) and trabecular number (Tb.N) were significantly decreased, while trabecular separation (Tb.Sp), percent osteoclast surface perimeter (%Oc.S.Pm), percent labeled perimeter (%L.Pm) and bone turnover rate (BFR/BV) were significantly increased in CIA rats. Poor trabecular structure and less trabecular bone of femur were seen in CIA rats by micro-CT scanning. BMD and biomechanical properties of femur were significantly decreased in CIA rats. Serum level of PINP, sRANKL and CTX-I were increased in CIA rats treated with vehicle for 90 days. Compared with CIA rats, %Tb.Ar and Tb.N of LV4 in CIA rats treated with prednisone for 30 days were increased, but there was no significant change in CIA rats treated with prednisone for 90 days. Prednisone treatment for 30 days increased bone volume and improved microarchitecture of distal femur. Prednisone treatment for 90 days increased bone volume, but there was no significant improvement in microarchitecture of femur. In additional, serum levels of OPG and PINP were decreased in CIA rats treated with vehicle for 90 days. There was no significant change in biomechanical properties of femur between CIA rats treated with vehicle and prednisone.
Discussion and Conclusion: High bone turnover osteoporosis could be shown in CIA rats, manifested by decrease of trabecular bone mass, structural degradation. Reduction of bone mineral density and biomechanical weak-ness. Prednisone treatment for 30 days decelerated the degeneration of trabecular bone in CIA rats, but did not improve bone mineral density and bone biomechanics. In additional, the protective effect of prednisone treatment for 30 days on bone in CIA rats was better than that of 90 days. Decrease of bone mass was not directly seen in CIA rats treated with prednisone for 90 days, but there was a negative effect on bone metabolism.
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Session: Biomechanics — Cell & Molecular Biomechanics

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PROMOTING EFFECT OF STRONTIUM ON THE DIVISION OF STEM CELLS IN BONE FORMATION

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Introduction: Strontium ranelate has been shown to reduce the risk of vertebral fracture in postmenopausal women [1]. The beneficial effects of strontium on promoting bone formation are closely related to its capability to increase the osteogenic differentiation of mesenchymal stem cells [2,3]. However, the molecular mechanisms are still not fully understood. The aim of this study is to investigate the mechanism underlying the promoted effect of strontium in inducing osteogenic differentiation of mesenchymal stem cells in early stage.
Subjects and Methods: Eight-week-old male C57BL/6 mice were randomly divided into experiment group and control group. Sr was daily oral administration in experimental group and vehicle (0.9% saline) in control group respectively for 4 weeks. The effect of Sr on trabecular bone microstructure was analyzed by micro-CT on proximal tibia. Human fetal BMSC (hfBMSC) which characterized by multi-differentiation were cultured in control group (control), osteogenic group (Osteo) and Sr promoted osteogenic group (Osteo+Sr). The osteogenic and asymmetric differentiation related genes were analyzed by quantitative PCR. The protein level of asymmetric differentiation related gene was detected by western blot. The effect of Sr on trabecular bone microstructure was analyzed by micro-CT on proximal tibia.
Results: The microstructure analysis of trabecular bone in proximal tibia showed that Sr significantly increased trabecular number and connectivity density. In the first week of hfBMSC culture, the related osteoblastic genes Osterix, osteopontin and BSP were significantly enhanced in osteogenic induced group from day3. With the addition of Sr, these genes were expressed at even higher levels. The gene Numb associated with asymmetric differentiation was promoted in Osteo+Sr groups in day3. At the same time in the immunofluorescence images, the osteogenic differentiation could be observed in Osteo and Osteo+Sr group. Oster+Sr showed higher proportion of the osterix positive cells. It should be noted that the divided cells showed one osterix positive cell and one oct-4 positive cell. In western blot, Sr stimulated the expression of Numb in the early stage of osteogenic differentiation.
Discussion and Conclusion: During the osteogenic differentiation of hfBMSC, Sr is related to the enhanced effect in asymmetric division of stem cell in the early stage.
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Session: Disease & Treatment — Osteoporosis

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DISRUPTION OF GLUCOCORTICOID SIGNAL IN OSTEOBLASTS AND OSTEOCYTES ATTENUATES HINDLIMB UNLOADING-INDUCED BONE LOSS IN MICE

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Objective: Bone loss induced by weightlessness has been a hot issue in space physiology, but the accurate mechanism is still not clear. At present, the lack of mechanical stimulation is considered to be an important factor for bone loss under weightlessness environment. However, besides mechanical unloading, it is inevitable that the stress in weightlessness environment induced increase of endogenous glucocorticoid (GC) levels. High levels of GCs have been established as risk factors for low bone mineral density and an increase in fracture risk. A recent progress showed that osteoblast-targeted disruption of intracellular GC signaling can prevent transgenic mice in chronic stress from bone loss, which indicated GC play an important role in stress-induced bone loss and a possible role in weightlessness induced bone loss. Therefore it is the aim of the present study to explore the role of GC in unloading-induced bone loss of mice.

Methods: We used the Col2.3-11β-HSD2 transgenic (Tg) mouse in which the GC-inactivating enzyme, 11β-hydroxysteroid-dehydrogenase type 2, is overexpressed in osteoblasts and osteocytes, resulting in disruption of intracellular GC signaling in these cells. 14-week old Tg mice and their wild type (WT) littermates were unloaded by tail suspension for the duration of 4 weeks.
Results: Unloading resulted in tibia loss of cortical bone in WT but not Tg mice when compared to non-unloaded controls. This was mainly due to a decrease in cortical area (Ct.Ar), cortical volume, mean total cross-sectional tissue area (Tt.Ar) and mean total cross-sectional tissue perimeter (Tt.Pm) in WT mice only. Trabecular bone in the tibia was similarly affected in WT and Tg mice by unloading. However, there are only significant effects on WT mice in the tibia cancellous bone of bone volume fraction (BV/TV), trabecular thickness (Tb.Th) and trabecular pattern factor (Tb.Pf). Moreover, the maximum force (F_{max}) and polar moment of inertia (pMOI) of tibia were significant reduced in WT mice after hind limb unloading, but no change in Tg mice. The ashing data in lumbar (L5) showed a significant decrease in wet weight, dry weight and ash weight of WT mice, but not in Tg mice. Meanwhile, the effect of hindlimb unloading on trabecular bone of the lumbar vertebrae was similar to the tibia in WT and Tg mice.
Conclusion: These results indicated that GC play a role in hindlimb unloading-induced bone loss of mice. Above all, the effect of GCs on cortical bone was more significant than that of cancellous bone in the bone loss induced by hindlimb unloading. The results above offered a new mechanism for the unloading-induced bone loss in mice.
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Session: Disease & Treatment — Osteoarthritis

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EFFECT OF COMBINATION SALVIANOLATE AND PREDNISONE THERAPY ON BONE HISTOMORPHOMETRY AND BIOMECHANICS IN COLLAGEN-INDUCED ARTHRITIS RATS

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Background: Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease with clinical manifestation of erosive and symmetric poly-arthritis. The combination therapy with prednisone and other drugs is now a research focus, but there's rare report about the effect of the combined application of salvianolate and prednisone on bone metabolism. We established the rat model of rheumatoid arthritis (RA) induced by type II collagen, and investigated the effect of combination therapy on bone metabolism in rats with collagen-induced arthritis (CIA).
Subjects and Methods: Forty-six 8-week-old female Lewis rats were randomly divided into 2 groups, control group with 6 rats and the remaining 40 rats were used to establish the CIA model. Four weeks after immunization, screening 27 CIA rats which successfully infected with arthritis (arthritis index≥4) were randomly divided into 4 groups. Rats in control group were given vehicle as well as in CIA group, while in the other groups were treated with prednisone at 4.5mg/kg/d or/and salvianolate at 20mg/kg/d. Drugs were administrated for 90 days. After sacrificed, the femur of rats was collected for bone biomechanical properties assay. The proximal tibial metaphysis (PTM) of rats was performed for histomorphometric analysis.
Results: Biomechanical properties (elastic load, maximum load, break load, stiffness coefficient) of femur in CIA group were significantly decreased compared with control group. Compared with CIA group, biomechanical properties (maximum load, break load and stiffness coefficient) were increased in CIA+PDN+Sal group, but there were no significant changes in CIA+PDN group and CIA+Sal group. Poor trabecular structure and less trabecular bone of PTM were seen in CIA rats. Compared with CIA group, percent trabecular area (%Tb.Ar) and trabecular number (Tb.N) in CIA+PDN group and CIA+PDN+Sal group were increased. Furthermore, %Tb.Ar and Tb.N in the treatment of salvianolate and prednisone group were lower than those in combination therapy group.
Discussion and Conclusion: Using prednisone to treat CIA ameliorated the cancellous bone loss of tibia, but did not improve biomechanical properties. Using combination prednisone and salvianolate therapy to treat CIA that was better to ameliorate the cancellous bone loss of tibia, and significantly improve biomechanical properties. The protective effect of combination salvianolate and prednisone therapy on bone loss in CIA rats was greater than that of prednisone and salvianolate alone.
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Session: Disease & Treatment — Pharmaceutical Interventions

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ICARIIN AUGMENTS BONE FORMATION THROUGH ACTIVATION OF CANONICAL Wnt SIGNALING PATHWAY

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Background: Traditional Chinese herbal medicine has been widely used for thousands of years for the treatment of bone diseases. Icariin belong to a class of flavonoid which is used to prevent bone loss and can be extract from several species of plants in the Epimedium family commonly known as Yin Yang Huo. In some reports, Icariin has been mostly reported to boost bone fracture healing and treat postmenopausal osteoporosis in ovariectomized animal model. Wnt signaling pathway with a foundational role during embryogenesis and normal cell development, which can regulates bone development and gene expression in whole process of bone metabolism. In this manuscript, we want to identify the role of icariin via regulating Wnt signaling pathway in bone formation.
Subjects and Methods: Alizarin red staining was used to estimate matrix calcification of mesenchymal stem cells in osteogenic differentiation basal medium compared to the same medium supplemented with icariin. Immunofluorescence test to detect expression of P-GSK in cytoplasm and localization of β-catenin in nuclear. Western blot were used to test expression of proteins of Wnt signaling pathway. Real-time PCR (RT-PCR) were used to test related osteogenic gene expression.
Results: Icariin added in osteogenic medium compared with normal osteogenic medium could promote the formation of calcium nodules in number and morphology by alizarin red staining. RT-PCR showed that Icariin could upregulate expression of osteogenic genes RUNX2, OCN, OSX. The result of western blot suggested that icariin could upregulate expression of P-GSK and active β-catenin. The result of immunofluorescence suggested that icariin could upregulate expression of P-GSK in cytoplasm and boost β-catenin expression in nuclear.
Discussion: Icariin, a flavonoid isolated from Epimedium family, has previously been identified to exert beneficial effects on preventing bone loss and promoting bone regeneration. Meanwhile in skeletal development, Wnt signaling, which play progression, is implicated in and commitment lineage MSC a crucial role in adipogenesis. In Wnt signaling, and chondrogenesis, osteogenesis, myogenesis non-phosphorylated GSK play an important role in degradation of β-catenin. In this study, these results have confirmed that icariin can promote the formation of calcium nodules in number and morphology, and upregulate phosphorylation of GSK so that inhibiting degradation of β-catenin in cytoplasm. With less degradation in cytoplasm, more β-catenin can into nuclear and play their bio-function.
Conclusion: In conclusion, icariin augments bone formation may via activate Wnt signaling pathway.
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Session: Biomaterials and Implants—New (Bio)materials

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A NOVEL CLAY-BASED NANOCOMPOSITE HYDROGEL WITH ATTRACTIVE MECHANICAL PROPERTIES AND ITS APPLICATION FOR BONE TISSUE REPAIR

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Introduction: In our study a novel nanocomposite hydrogel (NC gel) was successfully prepared by in situ free-radical photo-polymerization of the acrylic acid derivatives, macromolecular crosslinker (PEGDA) and nano-clay. The obtained hydrogel exhibits dramatic improvements in mechanical properties and the pre-in vivo test shows that this kind of novel hydrogel can accelerate bone formation [1—3].
Subjects and Methods: The NC gel was synthesized by in situ free-radical photo-polymerization of acrylic acid derivatives (monomer) and PEGDA (macromolecular crosslinker) in the presence of exfoliated clay. The clay content was varied from 1 to 10 wt % with respect to the monomer weight, and the solid content of the nanocomposite hydrogel varied from 20% to 30%. Characterization was carried out by tensile tests, compression tests, XRD, SEM and TEM. Pre-in vivo bone formation was studied using a rat bone defect model system.
Results: Mechanical tests show that the obtained novel clay-based nanocomposite hydrogel has the best tensile strength (about 800 kPa) and excellent stretch ability (higher than 5000%) when clay content and solid content are 5% and 20% respectively. The compression strength of the hydrogel is higher than 10 MPa