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03-P007 Study on bone formation in *Ano5*-knockout mice

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Gnathodiaphyseal dysplasia (GDD; OMIM#166260) is a rare skeletal disorder with autosomal dominant inherit pattern. It is characterized by lesions of jawbones, thickening cortical diaphysis of tubular bones and frequent fractures as a results of minimal injury. We found previously that the silence of Ano5 gene lead to increased mineral nodule formation in differentiating MC3T3-E1 osteoblast precursors in vitro and findings suggests that ANO5 plays a role in osteoblast differentiation. However, the pathological effects of ANO5 deficiency on GDD in vivo has not been elucidated completely. Now we generated a *Ano5*- konckout mouse modal with CRISPR/Cas 9 method. The expression of *Ano5* in bone tissue decreased significantly and some clinical features of human GDD have been replicated. Meanwhile, the mouse calvarial osteoblast (mCOBs) cultures was performed and the expression of osteoblast-related genes as well as bone matrix formation assays were investigated by quantitative PCR and alizarin red staining. The results showed that *Osteocalcin, Col1a1*, *Runx2*, *Osterix*, *Osteopontin* and *Rankl* highly elevated and mineralization enhanced drastically in $Ano5^{KO/KO}$ mCOB. The data are consistent with the achievements we observed before in vitro. We believe this new mouse model can contribute to he research into the pathogenesis of skeletal abnormalities in GDD and provide the clues to develop the therapeutic approaches for GDD.

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03-P008 A Biomimetic 3D Scaffold for Long Bone Repair

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It's a big challenge to cure long bone defects for bone tissue engineering in clinical therapy. In our study, we designed a biomimetic 3D scaffold with PLGA porous scaffold as inner layer and PLGA/HA nanofibers spiraled outside for long bone repair. Rabbit radius defects model with critical defect size of 15 mm was used to evaluate bone repair effects of the 3D scaffolds we designed. According to the implantation, rabbits are divided into five groups: (1) blank control without any implantation (control), (2) PLGA porous scaffolds (porous), (3) PLGA porous and nanofibers composite scaffolds (composite), (4) porous scaffolds seeded with rabbit bone marrow derived mesenchymal stem cells (porous/MSCs), and (5) composite scaffolds seeded with MSCs (composite/MSCs). The SEM results showed that the porous layer of PLGA had high porosity with pore size of 450 μm, and the diameter of PLGA nanofibers ranged from 150 nm to 270 nm. At week 4, 8, 12, and 16, it can be seen from X-Ray images that porous/MSCs group showed the best bone regeneration potential following with composite group. The micro-CT results showed the morphology and cross-section images of regenerated bones in each group, which indicated the bone regeneration and re-union. H&E and immunofluorescence staining results showed that more micro blood vessels appeared in composite group and porous/MSCs groups after implanted 4 weeks, which indicated the importance of new blood vessels for initial bone regeneration. SEM images of new bone at week 4 showed collagen formation and calcium deposition in composite group. The mechanical test of regenerated bones from each group showed that porous/MSCs group has similar mechanical property with normal bones. Taken together, we think MSCs could enhance the angiogenesis at the early stage of bone defection and PLGA porous scaffolds seeded with MSCs is one potential candidate for bone regeneration.