Effect of Scaffold Microstructure on New Bone Formation

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Statement of Purpose: Scaffold is a key component in bone tissue engineering that serves as a template for cell ingrowth and extracellular matrix (ECM) deposition. Porosity and pore size of the scaffolds play a critical role in bone formation in vitro and in vivo. The 3D architecture and geometry of the porous microstructure are characterized by pore size, shape, interconnectivity, and anisotropy. The design of the scaffold microstructure affects the tissue regeneration process as well as initial biological and biochemical properties. Previous studies have shown distinct microstructure in scaffold may influence cell seeding efficiency, cell migration, nutrition supply and waste removal. As a result of all these effects, we hypothesize that the structure of the new generated bone would also be influenced by the microstructure of the scaffold. To test this hypothesis, two different types of collagen/apatite scaffolds were made: cellular structure and lamellar structure. A double-hole mouse calvarial defect model was used to evaluate the influence of scaffold microstructure on the calvarial defect repair. Transgenic mice harboring GFP reporters for early-stage osteoblasts (pOBCol3.6GFPcyan and topaz) were used to differentiate the contribution of host/donor cells to new bone formation.

Methods: A double-hole mouse calvarial defect model was used to evaluate the effect of scaffold structure on new bone formaiton. The scaffolds were cut into disks of 3.5 mm in diameter and 1.0 mm in thickness. Calvarial osteoprogenitor cells (OPC) were derived from the calvariae of new born pOBCol3.6GFPcyan transgenic mice. The scaffold disks were loaded with 1 M osteoprogenitor cells from pOBCol3.6cyan transgenic mice. Two 3.5 mm critical-size calvarial defects were created in transgenic mice carrying pOBC3.6tpz transgene. The left side was implanted with lamellar structure scaffold (LSS) loaded with 1 M OPC, and the right side was filled with cellular structure scaffold (CSS) loaded with 1 M OPC. The mice were divided into 2 groups randomly and allowed to heal for 2 weeks or 4 weeks. All the mice were given a xylene orange injection 24 h prior sacrifice. The bone defect repair was assessed by radiological examination. The calvarial tissues were embedded and frozen-sectioned with a tape transfer system. The calvarial tissue sections were examined on a Zeiss Axio Imager Z1 equipped for fluorescence imaging and a computer-controlled mechanical stage for GFP imaging analysis. Hematoxylin and eosin (H&E) staining were also employed to analyze the new bone formation.

Results: Distinct scaffold microstructure was revealed by SEM as shown in Fig. 1. The LSS scaffold demonstrated an ordered layered structure with laminar distance of approximately 10 μm, where the CSS scaffold illustrated a typical cellular structure with pore size between 50-100 μm. X-ray radiographs showed new bone was formed even only after 2 weeks of implantation. It was found LSS formed more bone than CSS at this time point. More bone formation was observed at 4 weeks and no difference was detected between two scaffolds by X-ray imaging (Fig 1&2). Fluorescence microscopy observation showed most OPC concentrated at the bottom part of both scaffolds after 2 weeks of implantation, but new bone formation was only found in LSS at this time point. At 4 weeks, it was found both defects were filled by newly formed bone derived from donor OPC (Fig 3). It is important to notice that the new bone structure varies significantly with the scaffold microstructure. The bone formed in LSS demonstrated a typical lamellar structure which is similar to the scaffold structure. The new bone formed in CSS scaffold also followed the structure of the cellular scaffold. All the new bone formed during the healing process was contributed by donor cells. HE staining results was consistent with GFP analysis. Heavy fibrous tissue filtration was observed in both types of scaffolds, but no inflammation reaction was found either after 2 or 4 weeks.

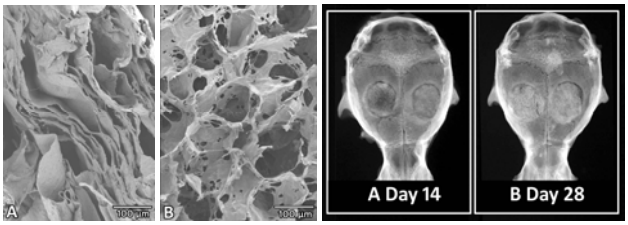

Fig.1&2 FESEM images of scaffolds with different microstructures (A) LSS; (B) CSS, and *X-ray micrographs of the defects at 2 weeks and 4weeks of implantation.*

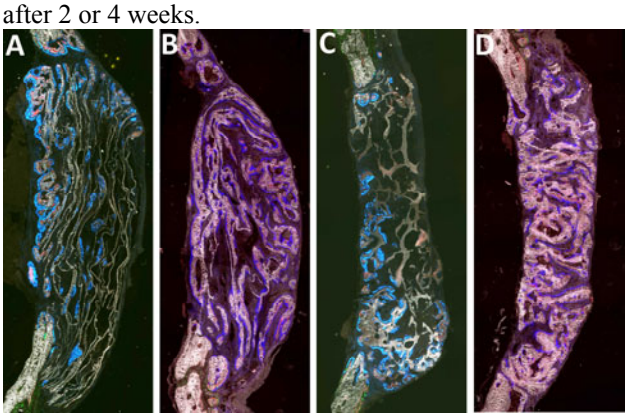

Fig. 3 Fluorescent imaging analysis of the cross section of the calvarial defects at different time points: (A)&(B) LSS; (C)&(D) CSS

Conclusions: New bone formation was observed in both LSS and CSS scaffolds which was verified by results from radiological examination and histology analysis. Scaffold microstructure has significant effect on guiding new bone formation. The adaption of the new bone to the scaffold structure demonstrates the remarkable guidance of scaffold to new bone growth.