Bone regeneration using collagen scaffolds loaded with different types of donor cells

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Statement of Purpose: There are more than 1 million cases of skeletal defects a year that requires bone-grafting procedures. Bone tissue engineering is a new emerging field, which has been proven to be a promising approach for bone defect repair [1]. It involves a combination of a scaffold matrix, bone cells and/or osteoinductive agents to form the tissue engineering construct. As one of the three key components of bone tissue engineering, the cell source for bone repair is extraordinarily critical. Qualified cell source should be easily expandable, non-immunogeneic and have a protein pattern similar to the tissue to be regenerated. Osteoprogenitor cells (OPC) are one of the few cell populations that can be derived from MSCs [2]. Bone marrow cell (BMC) is another practical cell source which has been used for bone regeneration [3]. In this study, we evaluated the osteogenic property of collagen/apatite scaffolds loaded with different cell populations. The effect of cell source on bone defect repair was studied in a mouse calvarial defect model. The contribution of donor/host cells to bone formation was differentiated using transgenic mice and GFP reporters.

Methods: Collagen/apatite scaffolds were prepared by a new one-step in situ co-precipitation technique where apatite nanoparticles and collagen nanofibers were simultaneously precipitated from a modified simulated body fluid (mSBF) at a mild temperature and pH. A double-hole mouse calvarial model was used to evaluate the osteogenic property of the scaffold loaded with different cell populations. Two 3.5 mm diameter defect was created at each side of the parietal bone at the calvarial site. Two groups of studies were carried out, and four mice in each group were tested. In the first group, the control (left side) was the scaffold alone, while the test was the scaffold loaded with pOBCol3.6cyan calvarial OPC. In the second group, the control was the scaffold loaded with pOBCol3.6tpz BMC, and the test was the scaffold loaded with calvarial pOBCol3.6cyan OPC plus fresh BMC carrying pOBCol3.6tpz transgene. The mice were subjected to a total body irradiation before implantation to suppress rejection of cells. They were then rescued with bone marrow transplantation, where the pOBCol3.6tpz and non-transgenic bone marrow cells were used in group 1 and 2 test, respectively. All the mice were sacrificed after 4 weeks of implantation. The bone defect repair was assessed by radiological examination. The calvarial tissues were embedded and frozen-sectioned using a tape transfer technique. 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 and tartrate-resistant acid phosphatase (TRAP) staining were also employed to analyze new bone formation and differentiate host/donor cell contribution.

Results: After 4 weeks, X-ray radiographs showed new bone formation in both groups. In the first group, GFP scanning analysis indicated that better bone formation was found in the defect filled with scaffold loaded with OPC. It was also found that the new bone was contributed by the donor cells. The scaffold loaded with OPC was completely resorbed and TRAP staining indicated strong osteoclast activity in the defect area (Fig 1). HE staining showed that host cells had migrated into the scaffold on both sides, but they were mostly fibroblasts instead of osteoblasts. In the second group, a host derived bone ingrowth was observed in the defect area loaded with BMC. Meanwhile, pOBCol3.6tpz positive cells were also found in the defect. In contrast, host derived new bone was not found in the defect loaded with BMC plus OPC. GFP analysis showed that the new bone was mainly derived from donor pOBCol3.6cyan cells when the defect was loaded with BMC plus OPC. The scaffold remained intact when new bone was not formed. HE staining results showed that the newly formed bone was connected with the host bone through a thick fibroblast layer.

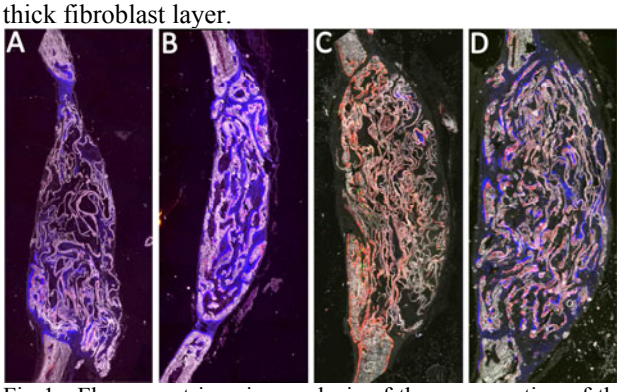

Fig 1. Fluorescent imaging analysis of the cross-section of the calvarial defects at day 28: A) Control; B) OPC; C) BMC; D) OPC+BMC

Conclusions: This study reports a novel double-hole mouse calvarial model used to evaluate the new bone formation activity on a collagen/apatite scaffold. Donor dominated bone regeneration was archived when OPCs present in the scaffold. When BMCs were loaded onto the scaffold, host-derived bone was formed. There was less bone formed but better integrated with the host comparing to the defect loaded with either OPCs or a combination of OPCs and BMCs. In contrast, the host did not contribute to new bone formation when BMCs were mixed with OPCs. It has been demonstrated that transgenic mice harboring GFP reporters for osteoblasts is a powerful tool to appreciate the host/donor contribution during bone repair.

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