Cell-sheet-engineered periosteum-like membrane promotes vascularization and osteogenesis of a β-TCP scaffold

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Statement of Purpose: Many studies have used synthetic bioceramic scaffolds for the repair of large bone defects [1]. However, the success of synthetic bone grafts is limited due to insufficient vascularization in addition to osteoinductivity, and osteogenesis *in vivo* once implanted [2]. To overcome this problem, several approaches were developed to increase the rate of vascularization in a scaffold after implantation, including the loading of growth factors (such as VEGF, PDGF) on an implanted bone scaffold [3], monoculture of endothelial cells, and co-culture of endothelials cells and bone-forming cells. However, these approaches still suffer from insufficient neo-vascularization in constructs and a slow invasion rate of host vasculature *in vivo* for large bone grafts. Therefore, a new robust strategy for improving rapid vascularization and functional anastomoses remains highly desirable. Periosteum is a membrane that covers the outer surface of bones. Studies have indicated that periosteum can increase the rate and quantity of bone formation and improve the vascular invasion ability in large segmental defects. If a vascularized periosteal sleeve is present, greater vessel invasion and more rapid bone formation in an implanted bone graft can be achieved. Cell sheet engineering has the potential to construct a tissue-engineered periosteum with its native structure. Therefore, in this study we proposed a new strategy for boosting vascularization and bone formation ability of the implanted bone graft through the combination of a cell-sheet-engineered periosteum (CSEP) and biodegradable porous beta-tricalcium phosphate (β-TCP) scaffolds. We hypothesized that the pre-vascularized biomimetic CSEP can promote neo-vascularization and new bone formation in the porous β-TCP scaffold and that the porous scaffold can support cell sheet transportation and implantation.

Methods: The CSEP contains an outer highly pre-vascularized cell sheet layer and an inner osteogenic cell sheet layer. The pre-vascularized cell sheet was formed by culturing human umbilical vein endothelial cells (HUVECs) on human mesenchymal stem cells sheet (hMSCs) and the osteogenic cell sheet layer was formed by inducing differentiation of hMSCs to mineral tissue. The two cell sheets were further sequentially wrapped on biodegradable β-TCP macroporous scaffolds to form periosteum-bone-like grafts (Figure 1a). To investigate the vascularized pattern of HUVECs on the hMSCs sheet, immunofluorescent staining of CD31 was performed. SEM was used to observe cell migration from the cell sheet into the scaffold. The periosteum-bone-like grafts were surgically implanted subcutaneously in nude mice for *in vivo* study. After 2, 4, and 8 weeks, the mice were euthanized and the implants were retrieved, decalcified, embedded, and sectioned. Conventional hematoxylin and eosin (H&E) and immunohistochemistry staining were carried out on the sections.

Results: *In vitro* studies indicated hMSCs promoted the pronounced formation of networks and lumens of HUVECs (Figure 1b). With time, cells migrated from the peripheral cell sheets into the struts of the β-TCP scaffold core. Histological evaluation results indicated that CSEP promoted angiogenesis *in vivo* (Figure 1c) and rapid functional anastomoses between the *in vitro* pre-vascularized human vascular networks and the mouse host vasculature system (Figure 1d). MicroCT analysis and osteocalcin staining indicated more mineralized bone matrix formation in the CSEP group compared to other non-periosteum-like groups (Figure 1e). Tartrate-resistant acid phosphatase staining showed osteoclast activity in the pre-vascularized cell sheet groups (Figure 1f). These results suggest that a periosteum-like cell sheet layer plays an important role in vascularization and osteogenesis of tissue-engineered bone.

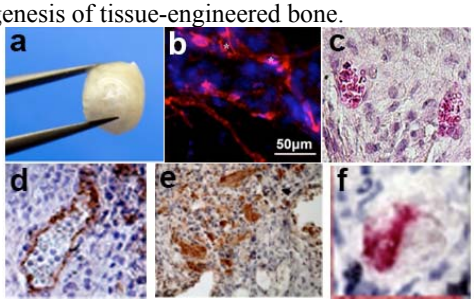

Figure 1. (a) The gross view of a CSEP/β-TCP graft. (b) hMSC sheet promoted the formation of vessel networks of HUVEC. (c) H&E staining shows the formation of blood vessels *in vivo* and functional perfused vasculature (d). Immunohistochemistry staining shows the deposition of bone mineralized matrix (e) and osteoclast activity (f).

Conclusions: Here we demonstrated a promising approach of engineered bone by integrating a periosteum-like 3D membrane based on cell sheet engineering, and and an interconnected, porous, biodegradable β-TCP scaffold. This *in vitro* pre-vascularized periosteum-like construct promoted the vascularization and osteogenesis of β-TCP scaffolds *in vivo*. The cell sheet-ceramic complex with periosteum/bone-like structure thus provides a promising candidate not only to biomimic native vascularized periosteum and promotes vascularization, but also to promote the osteogenic potential of bone grafts.

References:

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