Multiple Channels in β-Tricalcium Phosphate (β-TCP) Scaffold Promote Craniofacial Bone Tissue Regeneration

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Statement of Purpose: Insufficient oxygen and nutrient supply to the cells, which resulted from insufficient branched vasculature formation in a porous beta-tricalcium phosphate (β-TCP) scaffold, leaded to low cell survival and hindered the bone regeneration[1]. Channels could potentially facilitate nutrient diffusion and vascularization[2]. Therefore, in this study we fabricated multiple channels in porous β-TCP scaffold and hypothesized that the structure of an array of multiple channels in the porous β-TCP scaffold can promote cell proliferation and differentiation of mesenchymal stem cells *in vitro,* also bone tissue formation *in vivo*.
Methods: Porous β-TCP scaffolds with and without multiple channels were prepared by using a template-casting method[3]. Human bone marrow mesenchymal stem cells were cultured on the scaffolds and their proliferation and osteogenic differentiation were characterized. Gene expression of mesenchymal stem cells on the scaffolds under static and dynamic conditions was performed. Scaffolds were then implanted into the calvarial bone defects in rats. Immunofluorescent staining of bone osteogenic markers was carried out to investigate the bone formation.
Results: *In vitro* cell experimental results show that multiple channels significantly promoted cell attachment and proliferation of human bone marrow mesenchymal stem cells (hBMSCs), stimulated alkaline phosphatase activity, up-regulated osteogenic gene expression, and also highly stimulated various mechanosensing markers such as focal adhesion kinase (FAK), filamentous actin (F-actin), and Yes-associated protein-1 (YAP-1) at both static and dynamic culturing conditions. The *in vivo* bone defect implantation results demonstrated more bone formation inside multiple-channeled scaffolds compared to non-channeled scaffolds. Multiple channels prominently accelerated collagen type I, bone sialoprotein (Bsp), osteocalcin (OC) protein expression. Fluorochrome images and angiogenic marker CD31 staining exhibited more mineral deposition and longer vasculature structures in multiple-channeled scaffolds compared to non-channeled scaffolds.

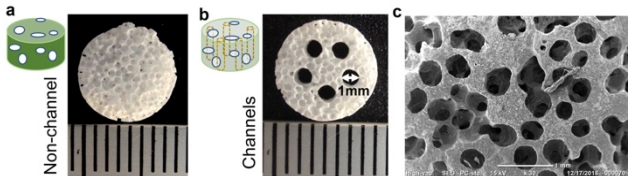

Fig. 1. Two types of β-TCP scaffolds: a) non-channeled porous scaffold; b) porous scaffold with five straight channels. The diameter of the channel is 1 mm; c) scanning electronic microscopic morphology of the porous scaffold.

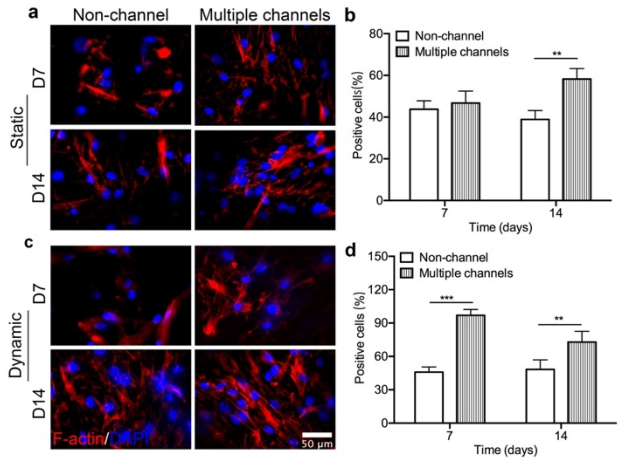

Fig. 2. F-actin staining of hBMSC cells on the two types of scaffolds under the static condition (a) and its positive signal rate quantification (b), and under the dynamic conditions (c) and its positive signal rate quantification (d) after 7 and 14 days. F-actin stain staining and its positive signal rate quantification show significantly more and stronger positive signals in the multiple channeled scaffolds (n=3, *p < 0.05, **p < 0.01, ***p < 0.001).

Conclusions: This study found that the creation of multiple channels inside interconnect porous β-TCP scaffold significantly increased its cell proliferation. Multiple channels enhanced osteogenic gene expression *in vitro*, and promoted bone formation *in vivo*. The interconnect channels-pore geometry in porous β-TCP scaffolds had significant functionality to stimulate new bone formation. The findings implied that the architecture of multiple channels-pores in the scaffold act as a promising stimulator to promote bone regeneration. This study suggested that multiple channel's geometry has promising potential to enhance bone formation *in vivo*.

References:

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