A Novel Approach to Engineer Vascularized Osteon-like Constructs for Cortical Bone Tissue Engineering

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Statement of Purpose: Bone tissue engineering has proved to be a promising alternative to autografts in repairing and regenerating large bone defects [1]. Compared to trebacular bone, one of the major challenges in cortical bone engineering is how to recapitulate the hierachical structure of cortical bones. We have proposed a bottom-up approach to assemble "osteon-like" building blocks into a complex and compact cortical-like bone structure. Here, we demonstrated the application of a novel two-step fabrication process to engineer the vascularized osteon-like structures. This approach combined electrospinning and twin screw extrusion/spiral winding (TSESW) techniques to generate osteon-like constructs with the electrospun nanofiber tubes as the core (Fig. 1A). Endothelial cells are seeded onto the nanofiber tube lumen surface to mimic the Haversian cannals and the polycaprolactone (PCL) / biphasic calcium phosphate (BCP) outer layers support bone tissue formation. This study firstly studied the feasibility of applying TSESW technique to make small-sized cylindrical scaffolds and investigated the effects of scaffold structures and composition on cellular responses.

Methods: TSESW [2] and electrospinning was used to fabricate the nanfiber/microfiber osteon scaffolds from PCL and BCP. Mouse preosteoblasts (MC3T3-E1) cells were seeded and cultured onto the TSESW scaffolds in spinner flask at stirring speed of 40 rpm. Media containing DMEM (Invitrogen, USA) supplemented with 10 % FBS and 1 % antibiotics was used for culture. The culture was harvested at Day 3, 7 and 14, washed with PBS and fixed with 4% paraformaldehyde for 2 h at RT. The fixed sample was embedded and cut into thin sections and then stained with hematoxylin and eosin for histological examination. Bioassays were done for cell proliferation and the expression of osteogenic markers (Ca deposition and ALP activity).

Results: It was found that the outer layers of scaffolds with media pitch size and BCP addition could favor the proliferation and differentiation of pre-osteoblasts (Fig. 1 B-I). Moreover, inner surface of central electrospun hollow tubes could be lined with a single layer of endothelical cells (ECs, MS-1) to form vessel walls surrounding lumens (Fig. 1 J-L). Therefore, this two-step fabrication process serves as a novel and promising strategy for engineering of osteon analogs.

Conclusions: This finding would provide us an innovative avenue to engineer osteon-like structure by combining TSESW and electrospinning techniques. This engineered osteon-like constructs show potential for assembly into artificial cortical bones for future therapeutic replacements.

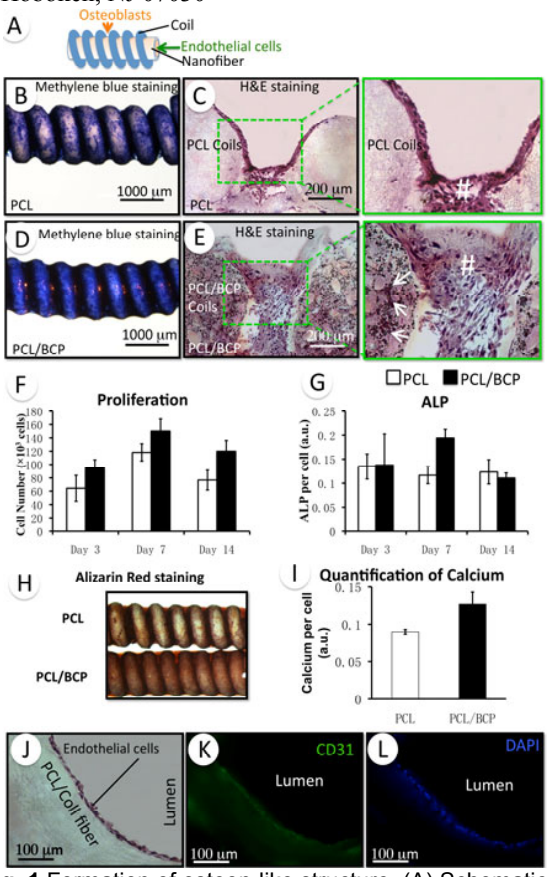

Fig. 1 Formation of osteon-like structure. (A) Schematic illustration of the scaffold design, in which PCL or PCL/BCP microfibers are spirally wound onto a PCL/collagen nanofiber tube to form a hybrid tubular structure. (B)-(E) Optical images of the hybrid scaffold cultured with MC 3T3-E1 cells for 7 days. (B) and (D) Stereo microscopic images of the culture stained with methylene blue. (C) and (E) Thin cross-sections of the culture stained with hematoxylin and eosin (H&E). PCL/BCP hybrid scaffolds (D and E) promoted tissue formation in between the interfiber space (#). BCP particles (arrows) were visible in the cross-sections. (F) Cell proliferation in the hybrid scaffolds as determined by DNA assay (n=4). (G) Normalized alkaline phosphatase (ALP) activity (n=4). (H) Representative image of alizarin red-stained osteon-like constructs, in which the hybrid scaffolds were cultured with preosteoblasts for 7 days. (I) Quantitative analysis of calcium deposition after alizarin red staining (n=4). (J)-(L) Seeding and culture of MS-1 cells on the PCL/collagen nanofiber lumen surface of the hydrid scaffolds for 3 days. (J) Thin cross-sections stained with H&E. (K) and (L) Representative immunofluorescent images of the cross-sections stained for endothelial cell marker CD31 (green) and cell nuclei (blue).

References: [1] Mistry AS and Mikos AG. Adv Biochem Eng Biotechnol. 2005;94:1-22. [2] Ergun A, Yu X, Valdevit A, Ritter A, Kalyon DM. J Biomed Mater Res A. 2011;99(3):354-66