Scaling-Up and Quality Assurance of Tyrosine-Based Polymer/Calcium Phosphate Scaffolds for Bone Regeneration in Sheep Tibial Model

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Statement of Purpose: The current standard treatment for long bone defects and complex fractures include wound management, fixation and bone grafting in both civilian and military practice, however, none of which has provided over 90% success.[1] Previously, composite scaffolds made from tyrosine-derived polycarbonate (E1001(1k)) and beta-tricalcium phosphate (β-TCP) were implanted into rat and rabbit craniofacial models and demonstrated good biocompatibility, biodegradability and osteoconductivity.[2, 3] In this study, long tubular composite scaffolds have been successfully scaled up and were tested in a 30 mm critical-sized defect (CSD) sheep tibial model. Custom-made punches and fixtures were designed to obtain the long and tubular shape of the scaffolds. In order to overcome the low bone regeneration of bone void fillers in critical size defects, the scaffolds were used as carriers for bone morphogenetic protein 2 (BMP-2) to enhance the osteoinductivity. A custom-made loading chamber was designed for the uniform loading of BMP-2 solution. Good manufacturing practice (GMP) was performed in the lab-scale production of scaffolds, the batch number and individual scaffold was tracked throughout the fabrication process.

Methods: E1001(1k)/β-TCP scaffolds were fabricated by a combination of porogen leaching and freeze-drying technique.[2] Custom-made punches and fixtures were used to shape 3 cm tubes. The pore structure was examined by Scanning Electron Microscope (SEM). The distribution of β-TCP was investigated by Thermal Gravimetric Analysis (TGA). The porosity was examined by Helium Pycnometry. Since the scaffolds were used as carriers for BMP-2, a loading chamber was design and fabricated to allow ultrafast and uniform loading prior to implantation.

Results: Photos of the scaffolds are presented in Figure 1A – 1C. The scaffold was immobilized on a surgical rod in the sheep tibial model. The SEM images were shown in Figure 1F, the porous scaffolds matrix is composed of macropores (212-450 μm) and micropores (20 μm) aligned along the walls of macropores. The pore structures were consistent in the top, middle and bottom parts. The porosity (90%) and distribution of β-TCP for top, middle and bottom parts were listed in Table 1. For the loading of BMP-2 solution, the semi-vacuum loading was examined by using a mixture of BSA and blue food dye solution. The even loading can be visualized by the blue color in Figure 1G.

Table 1 Porosity and β-TCP distribution of top, middle and bottom parts.

Part of scaffolds	Prorosity (%)	wt% of β-TCP
Top	91.03 ± 0.28	28.2 ± 0.2
Middle	91. 24 ± 0.71	24.9 ± 1.7
Bottom	91.34 ± 1.06	25.7 ± 1.0

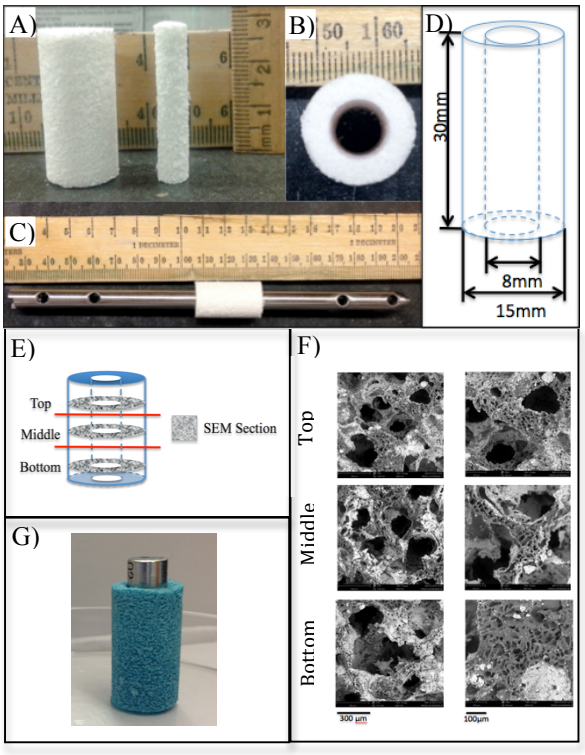

Figure 1 A) - C) Photos of E1001(1k)/ β-TCP scaffolds; D) illustration of the scaffold; E) horizontal sections of top, middle and bottom parts for SEM; F) SEM images; G) scaffold loaded with blue food dye.

Conclusions: The long, tubular composite scaffold can be successful fabricated by using a series of custom-made molds. GMP was performed during lab-scale fabrication for large animal study. The distribution of pore size and β-TCP was uniform throughout the scaffolds. A semi-vacuum loading chamber was successfully designed to obtained uniform loading of BMP-2 solution.

References:

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