Biomimetic Hydroxyapatite/Collagen Scaffold for Bone Repair

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Statement of Purpose: Human bone is a hybrid of hydroxyapatite (HA) nanoparticles and type I collagen nanofibers, which assembles into both the dense cortical bone (porosity= 3-12%) and highly porous cancellous bone (porosity= 50-95%).[1] The pores in bone are interconnect and aid the transportation of nutriment as well as the in-growth of new tissue.[1] Thus, porous HA/collagen scaffold becomes a prospective candidate for bone tissue engineering. In this work, a porous scaffold with interconnected pores was fabricated using a one-step in situ co-precipitation technique where HA nanoparticles and collagen nanofibers were simultaneously precipitated in a modified simulated body fluid (m-SBF). The bone forming property of the scaffold was evaluated in a novel mouse calvaria model and interpreted using green fluorescent protein (GFP) reporters.

Methods: Type I collagen was extracted from rat tail and was used to prepare collagen-contained m-SBF solution. The solution was kept at 40°C for 24 h to allow in situ co-precipitation of nano-HA particles and collagen nanofibrils. The precipitates were then collected and lyophilized to form a 3-D apatite/collagen composite scaffold. The composition and morphology of the scaffold were characterized using XRD, TGA and FESEM, respectively. A mouse calvaria model was used with two 3.5 mm defects created at each side of the suture line at the calvaria site. One positive control (Healos®, a commercially available apatite/collagen scaffold) and one test scaffold were implanted. The scaffolds were loaded with mouse calvaria osteogenic progenitor cells carrying the Col3.6GFPcyan transgene (donor cell, blue color) and implanted in a green host mouse (Col3.6GFPtpz). After 28 implantation days, the mice were injected with a mineralization dye (xylenol orange) one day prior to sacrifice and the calvaria with implants were harvested, embedded and frozen-sectioned. A full-size image of the bone section was reproduced by tiling together images obtained from a Zeiss Axiovert and AxioObserver work station.

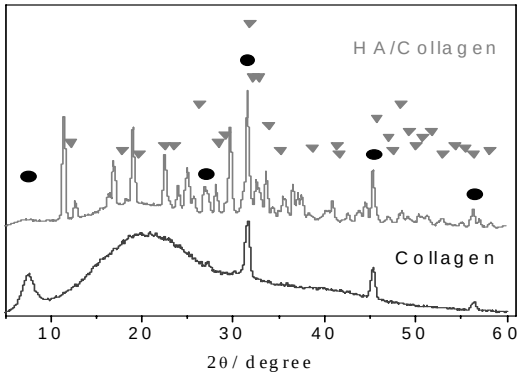

Figure 1. XRD patterns of collagen and HA/collagen co-precipitated scaffolds. ●: collagen peaks; ▼: HA (9-432) peaks.

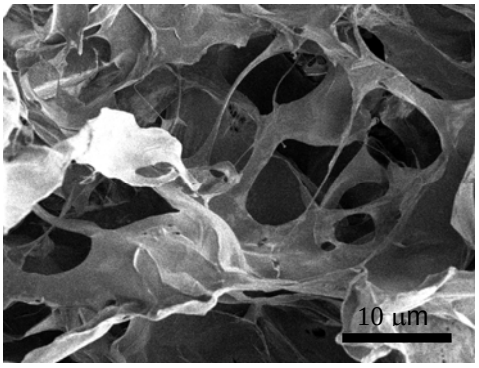

Figure 2. FESEM images showing the morphology of the HA/collagen scaffold.

Results: The studied scaffold was found to be a hybrid of HA and collagen by comparing its XRD patterns to those of collagen and of pure HA (9-432) (Figure 1). The HA content (24-57 wt%) and pore size are adjustable by varying the collagen content in m-SBF and water content in the precipitates, respectively. The scaffold demonstrated a 3-D interconnected porous structure (Figure 2). Both macro- and micro-pores are present in the scaffold, which will contribute to the transportation of nutriments as well as the formation of new tissue. To gain insight into its bone forming ability, the scaffold was evaluated using a novel mouse calvarial model. After harvested at 28 days of implantation, the implants were embedded and frozen-sectioned. The image of the bone section shows that new bone was formed in the defects with both control and test scaffolds, but more new bone was observed at the test site. Also, it was found that the new bone formation in both control and test were mainly contributed by donor cells which demonstrated blue color overlying a red mineralization line (Figure 3).

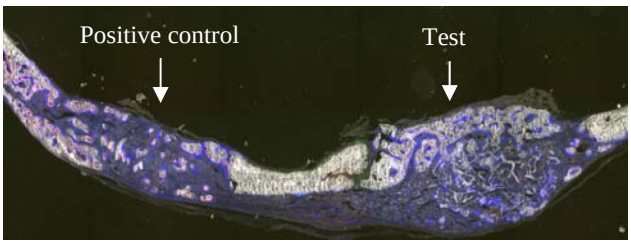

Figure 3. Cryosection of mouse calvarial defect repair after 28 days of the implantation.

Conclusions: HA/collagen composite scaffolds were prepared using an in situ co-precipitation method. The HA content and pore size of the scaffold can be tailored to meet different requirements. Such prepared scaffolds support osteoprogenitor cell attachment and proliferation as well as new bone formation in a mouse calvaria model. The new bone produced in the scaffold was mainly contributed by donor cells.

References:

1. Weiner, S. Annu Rev Mater Sci. 1998;28:271-298