In-Vivo Bone Formation in RGD-Conjugated Crosslinked Poly(Lactide) Scaffolds with Well-Defined Pore Geometry

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Statement of Purpose: There are 6.2 million fractures in the US annually that require bone graft procedures to ensure rapid skeletal repair and achieve union. Poly(lactide-co-glycolide) (PLGA) is the most widely used biodegradable polymer in the biomedical field1 which is FDA approved for a number of clinical applications. High molecular weight PLA, when functionalized with acrylate, methacrylate, or fumarate groups, do not crosslink to an appreciable extent due to the low density of reactive groups and slow chain diffusivity. We have developed novel short acrylate-terminated star PLA macromers (sPLAA) that can be formed into crosslinked scaffolds with well-defined pore geometry by rapid-prototyping. The objective of this work was to evaluate bone formation in crosslinked PLAA in-vivo.

Methods: star 6-arm short PLAA was synthesized by ring opening polymerization of the lactide (LA) using dipentaerythritol (DPE) as initiator. It is well established that the pore size and geometry affect the distribution and extent of extracellular matrix formation. Therefore, an FDM-3000 Fused Deposition Modeler (FDM) RP system was used to build porous sacrificial molds layer-by-layer using hot extruded wax laid down in 400 µm width struts, as described [1], and shown in Figure 1a (3-D image) and 1b (2-D cross sectional image by SEM). The finished mold was infused with sPLAA polymerizing mixture and allowed to crosslink. The construct was immersed in hexane to dissolve the wax, leaving just the crosslinked sPLAA scaffold behind [1]. Figure 1c shows the SEM image of the cubic scaffold (8x8x5 mm). Figure 1d shows the 3D reconstruction of a portion of the scaffold from the 2D µ-CT images

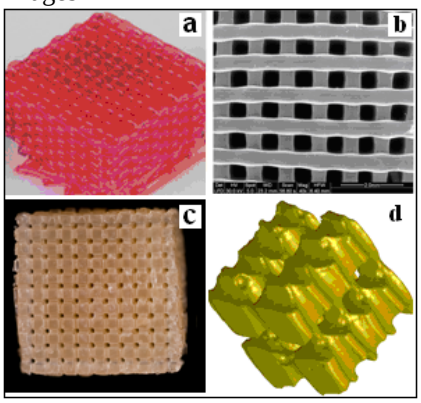

Figure 1. sPLAA scaffolds produced by Rapid Prototyping.

The last two images show the completely interconnected scaffold pore morphology. GRGD peptide was synthesized and acrylated directly in the solid-phase to produce Ac-GRGD peptide, as described [2]. Ac-GRGD peptide (1% by weight of sPLAA) was added to the polymerizing mixture to produce RGD-conjugated PLAA scaffolds with well-defined pore geometry. Bone formation was assessed in-vivo with sPLAA scaffolds in a bone defect (segmental femur) and an ectopic site (subcutaneous implantation) in rat animal model.

Results: By addition of glycolide (GL) to sPLAA macromer, the rate of scaffold degradation can be modulated to the rate of matrix formation, as shown in Figure 2a. The scaffold modulus, hence mechanical strength, can be improved by addition of apatite nanocrystals to sPLAA matrix, as shown in Figure 2b.

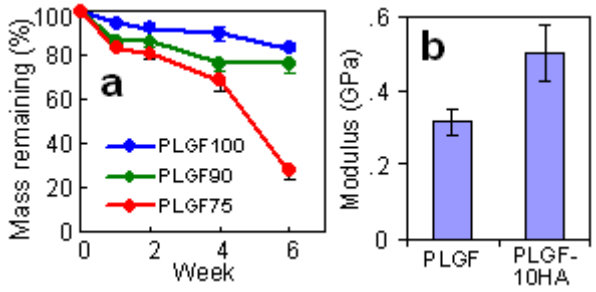

Figure 2. Degradation and Modulus of sPLAA scaffolds.

The implanted scaffolds were removed and stained with hematoxylin and eosin (H&E) for cell visualization. Excellent integration of the scaffold with the surrounding bone tissue and the marrow cavity was observed when implanted in the femur defect. Figure 3c and 3d show H&E sections of one of the pores of PLGF scaffold without and with dipping in rhBMP-2 solution (20 µg/ml) after 9 weeks of subcutaneous implantation. Furthermore, tissue formed in scaffolds without rhBMP-2 was fibrous while that with rhBMP-2 was dense lamellar bone.

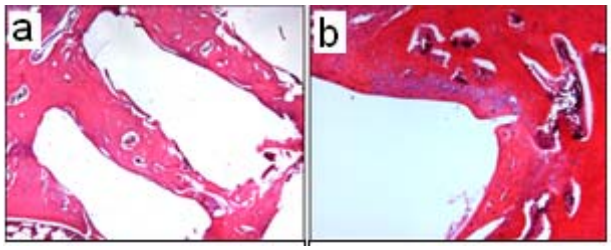

Figure 3. Explanted scaffold stained with H&E

Conclusions: In-vivo results demonstrate that RGD-conjugated sPLAA scaffolds support osteogenesis and bone formation.

Reference:

[1] E. Jabbari, D. Rocheleau, W. Xu, X. He, Proceed. ASME Inter. Conf. Manuf. Sci. Eng. (2007).
[2] E. Jabbari, X. He, M.T. Valarmathi, A.S. Sarvestani, W. Xu, J. Biomed. Mater. Res. A in Press (2008).