In Situ Crosslinkable Bioresorbable Poly(Lactide Fumarate) Scaffolds for Guided Bone Regeneration

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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. Current clinical methods of treating skeletal defects involve bone transplantation or the use of other materials to restore continuity. Poly(lactic acid) (PLA) and its copolymers with glycolic acid are FDA approved for certain orthopedic applications but these polymers require organic solvents or elevated temperatures for scaffold fabrication. Our laboratory has developed a novel unsaturated PLA-based macromer which can be injected and crosslinked in situ for applications in minimally invasive procedures in skeletal tissue regeneration. The objective of this work was to evaluate material properties and osteoconductivity of PLAF-based scaffolds.

Methods: Our laboratory has developed the following procedure to synthesize unsaturated poly(lactide fumarate) by polycondensation of low molecular weight (LMW) poly(L-lactide) (PLA) with fumaryl chloride [1]. In the first step, LMW PLA is synthesized by ring-opening polymerization of the lactide monomer in a dry glass ampoule with diethylene glycol (DEG) as the bifunctional initiator and tin octoate as the catalyst. In the second step, PLAF macromer is synthesized by condensation polymerization of LMW PLA with fumaryl chloride. For scaffold fabrication, PLAF was mixed with 20% 1-vinyl-2-pyrrolidinone (NVP) as the crosslinking agent and 10% methylene chloride (MC) as a diluent. 70% by volume sodium chloride salt crystals was added to the polymerizing mixture as the porogen. 50 µl per gram PLAF benzoyl peroxide (BP) solution as the initiator and 40 µl per gram PLAF dimethyl toluidine (DMT) solution as the accelerator was added to the mixture. The polymerizing mixture was transferred into a Teflon mold and placed in an oven for 30 min to facilitate crosslinking. After crosslinking, the salt was leached out by soaking the scaffolds in distilled water. The scaffold pore morphology was studied with an ESEM equipped with an electron backscattered detector. The PLAF scaffold was evaluated in vivo for bone formation by implantation in nude mice. Scaffold disks 8 x 3 mm were sterilized in excess 70% ethanol and implanted with seeded fetal bovine osteoblasts subcutaneously in the back of two nude mice. The load of the osteoblasts was 1.5 x 10^6 cells/implant. Eight weeks after implantation, the animals were sacrificed and samples were removed. Samples were demineralized, dehydrated in sequential ethanol solution, embedded in paraffin, sectioned and stained with H & E. Ostaeoblasts were isolated from 1-3 mm strips of immature bone taken from the femur and tibia of fetal calves. The bone strips were dispersed enzymatically using 0.5 mg/ml collagenase. Cells were collected from the supernatant by centrifugation and cultured at a density of 1x10^4 cell/cm^2 in complete media (DMEM with 10% FBS, 10 ml of 1% (v/v) antibiotic and antimycotic agents, 10-3 M β-glycerol phosphate, and 50 mg/l L-ascorbic acid). After reaching 70-80% confluency, cells were enzymatically lifted and loaded into scaffolds. The degradation characteristics of the PLAF scaffolds were determined in vitro in PBS at 37°C.

Results / Discussion: The cross and longitudinal sections of the scaffold after salt leaching, examined with SEM, are shown in Figures 1(a) and 1(b), respectively. The SEM micrographs in Figure show a highly porous and interconnected scaffold with 300 µm average pore size.

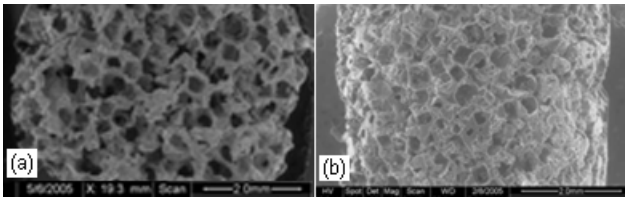

Figure 1. Cross (a) and longitudinal (b) sections of the scaffold.

Figure 2 demonstrates that greater than 50% of the scaffold mass was degraded after 6 month in vitro. A section of an explanted scaffold stained with H&E is shown in Figure 3. Significant amount of newly formed woven bone is seen. Blue-purple areas of cartilaginous tissue are also seen indicating a mode of endochondral bone formation. The void areas represent the residue scaffold materials which were dissolved during the process of histology preparation. This bone formation was seen on the explants harvested at both 6 and 8 weeks of implantation.

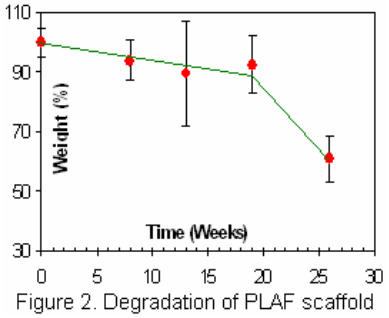

Figure 2. Degradation of PLAF scaffold

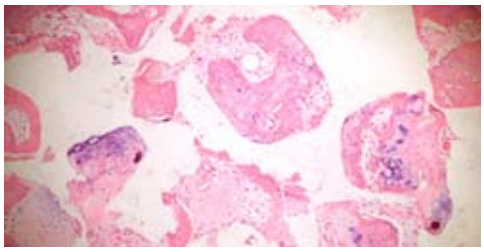

Figure 3. Explanted scaffold stained with H&E

Conclusions: The initial animal testing demonstrates that PLAF supports bone formation when seeded with osteoblasts. The PLAF scaffold is potentially useful in orthopedics for guided bone regeneration.

Reference: [1] Jabbari E, Synthesis and characterization of poly(L-lactide) networks as injectable scaffolds for guided tissue regeneration, Annual meeting of the AIChE, Cincinnati, OH (2005).