Analysis of the In Vivo Bone Forming Capacity of a Copolymer Consisting of Poly(propylene fumarate) and Poly(ε-caprolactone)

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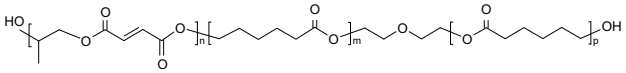
A novel copolymer poly(propylene fumarate-*co*-caprolactone) (PPF-*co*-PCL) with 15 compositions of PCL was invented (Scheme 1) to obtain controllable physical properties for various needs in tissue engineering, particularly, bone and nerve regeneration. The biodegradation rate and mechanical properties can be modulated by the copolymer composition and crosslinking density. In this study, we tested the *in vivo* biocompatibility of one PPF-*co*-PCL copolymer with a PCL composition of 31% [PPF-*co*-PCL (31%)] and explored its capacity to support bone formation.



Scheme 1

Methods: PPF-*co*-PCL was synthesized as described previously.[1] The weight-average molecular weight of PPF-*co*-PCL (31%) was 8230 g/mol and the number-average molecular weight was 4030 g/mol. PPF-*co*-PCL (31%)/BAPO/CH_2Cl_2 solution was mixed with salt (NaCl) particles (300-400 μm), the mixture was placed a glass mold and crosslinked under UV light for 30 min. Porous scaffolds (5 × 3.5 mm, length × diameter) with a porosity of 80% were obtained after salt leaching. Poly(lactic-co-glycolic acid) (PLGA) microspheres containing vehicle or recombinant human bone morphogenetic protein-2 (rhBMP-2) were mixed with PPF-*co*-PCL (31%)/BAPO/CH_2Cl_2, placed in a glass mold and crosslinked under UV light for 30 min. These scaffolds had ~6.5 mg PLGA microspheres with ~10.3 μg rhBMP-2 or vehicle in each implant. The scaffolds were implanted subcutaneously in male Sprague Dawley rats (weight 310-335 g) to evaluate biocompatibility and bone formation[2]. The scaffolds were excised, fixed in 70% ethanol and dehydrated through ascending concentrations of ethanol. After dehydrations the scaffolds were embedded in glycol methacrylate to facilitate sectioning of the mineralized tissue. The embedded scaffolds were sectioned longitudinally and 5 micron sections were collected for analysis at a depth of 50 to 200 microns from the face of the scaffold. Four sequential sections from each scaffold were stained with a modified Goldner's Trichrome stain in order to differentiate between unmineralized tissue and cells, which stain red, and mineralized tissue which stains green. Quantitative histomorphometry was performed using an OsteoMeasure system (OsteoMetrics, Decatur, GA). The parameters measured were: total scaffold area, percent of scaffold area containing cells, and the distance the cells had grown in from the vertical and the horizontal perimeter of the scaffold. StatView software was utilized for the statistical analysis and a Fisher's PLSD analysis was performed.

Results: The scaffolds were sectioned in a longitudinal plane until the full area of the rectangular scaffold was exposed and 4 sequential, 5 micron sections were cut for analysis. This region of interest corresponded to a depth between 50 to 200 microns from the face of the scaffold. The 2 D scaffold areas, mm^2 (mean ± SE), were similar for all scaffolds: PPF-*co*-PCL (8.7±0.4), PPF-*co*-PCL with microspheres (9.1±0.4), PPF-*co*-PCL with rhBMP-2 (9.0±0.5). The PPF-*co*-PCL scaffold had significantly greater area covered by cells (53.9±2.7%) compared to the PPF-*co*-PCL scaffold with microspheres (21.7±2.2%, $p<0.0001$) and the PPF-*co*-PCL with rhBMP-2 (42.8±4.1%, $p<0.05$). The addition of rhBMP-2 significantly increased the cell growth ($p<0.0001$) compared to the scaffold containing microspheres. The PPF-*co*-PCL group also had significantly greater cell in growth from the perimeter of the scaffold, (2.5±0.1 mm, horizontal, 1.9 ±0.2 mm, vertical) than the PPF-*co*-PCL with microspheres (0.4±0.04 mm horizontal, 0.3±0.02 mm, vertical) $p<0.0001$, or the PPF-*co*-PCL with rhBMP-2 (0.7±0.1 mm, horizontal, 0.4±0.02 mm, vertical) $p<0.0001$.

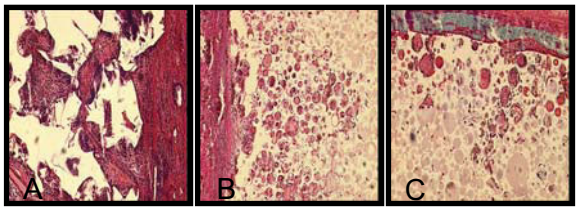


FIGURE 1 Goldners Trichrome staining of PPF-*co*-PCL (31%) Scaffolds. A) PPF-*co*-PCL (31%); B) PPF-*co*-PCL (31%) with microspheres; and C) PPF-*co*-PCL (31%) with rhBMP-2.

Figure 1 shows PPF-*co*-PCL (31%) in panel A had increased cell growth, while bone formation (green stain) occurs at the perimeter of PPF-*co*-PCL (31%) with rhBMP-2 in panel C.

Conclusions: The PPF-*co*-PCL (31%) was able to support cell in growth in an in vivo, subcutaneous model. The addition of microspheres to the PPF-*co*-PCL (31%) decreased the initial cell in growth; however, the microspheres were needed as method for delivering the rhBMP-2. As shown in Figure 1, the scaffolds containing rhBMP-2 were the only scaffolds able to support bone formation. Therefore, the PPF-*co*-PCL (31%) shows promise as a scaffold for bone regeneration, however, the method for incorporating and delivering rhBMP-2 from this scaffold needs further investigation.

References:

1. Wang SF, et al. Macromolecules 2005;38:7358.
2. Kempen DHR, et al. Tissue Eng A 2008; in press.

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