A Novel Injectable Polymeric Biomaterial Poly(propylene fumarate-*co*-caprolactone) with Controllable Properties for Bone and Nerve Regenerations

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Recently a novel crosslinkable and biodegradable copolymer poly(propylene fumarate-*co*-caprolactone) (PPF-*co*-PCL) has been synthesized in our lab (Figure 1). By varying copolymer composition, controllable physical properties can be achieved to satisfy various needs in tissue engineering, particularly, bone and nerve regenerations.[1] The physical properties of the copolymers have been extensively investigated and the glass transition temperature (T_g) decreases progressively with increasing the PCL composition.[1] Therefore, the biodegradation rate and mechanical properties can be well modulated by the different molecular structure, copolymer composition, and crosslinking density.

In this study, the crosslinking characteristics and biodegradation of PPF-*co*-PCL copolymers using both chemical crosslinking and photo-crosslinking methods have been investigated in detail. The shape-memory effect has been revealed when the composition of PCL is high enough to show the crystallinity. Furthermore, we have performed the biological evaluations of several PPF-*co*-PCL copolymers with different PPF compositions in order to explore their bone and nerve tissue-engineering applications.

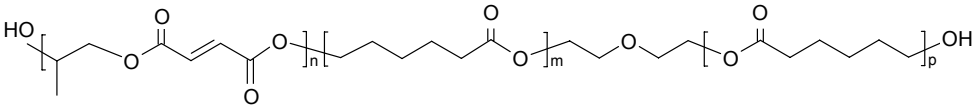


Figure 1. Chemical Structure of PPF-*co*-PCL.

Fifteen copolymers with a variety of PCL compositions ranging from 29 to 90% were used in this study and their weight-average molecular weights are between 8,230 and 47,100 $g.mol^{-1}$. In thermal-crosslinking process, benzoyl peroxide (BPO) and *N*-dimethyl toluidine (DMT) were used as the free radical initiator and accelerator, respectively. One hundred microliters of initiator solution (50 mg of BPO in 250 μL of NVP) and 40 μL of accelerator solution (20 μL of DMT in 980 μL of CH_2Cl_2) were added in 1.5 g PPF-*co*-PCL solution in 500 μL of CH_2Cl_2 and mixed thoroughly. The polymerizing solution was then transferred into Teflon molds and the molds were placed in a convection oven overnight to facilitate crosslinking. After crosslinking, the crosslinked samples were removed from the mold after they were cooled to ambient temperature. Photocrosslinking were initiated with UV light (λ=315-380 nm) using a photoinitiator bisacylphosphine oxide (BAPO). The homogenous mixture of polymer and BAPO in CH_2Cl_2 was transferred to a mold formed by two glass plates and a Teflon spacer. The mold was placed directly under UV light for 30 min to facilitate crosslinking. The gel fraction and swelling ratios in various solvents were determined. The thermal properties were measured by using a Differential Scanning Calorimeter (DSC) and a Thermalgravimetric Analyst (TGA). The mechanical properties including compression modulus, flexibility, surface rigidity, and pulling-out strength have been tested on a Dynamic Mechanical Analyst (DMA).

The copolymers were found to be both self-crosslinkable without using a crosslinker and photo-crosslinkable. The gel fractions of the photocrosslinked copolymer disks are close to 100%. The crosslinked copolymer disks are quite hydrophobic and little swelling can be detected in PBS solution. It can be seen in Fig.2a that only one broad glass transition exists for the crosslinked copolymers and T_g decreases with increasing PCL composition when the PCL composition is higher than 54%. No discernible glass transition was found for lower PCL compositions because of high crosslinking density. The crystallinity of PCL segments was suppressed significantly first by amorphous PPF segments and then crosslinks. Only the sample with a highest PCL composition of 90% shows a weak crystalline peak at 17.1 ^{o}C. Thermal degradation temperature increases from 332 to 390 ^{o}C when the PCL composition increases from 30 to 90%. The mechanical properties can be modulated by the copolymer composition, as shown in Fig. 2b.

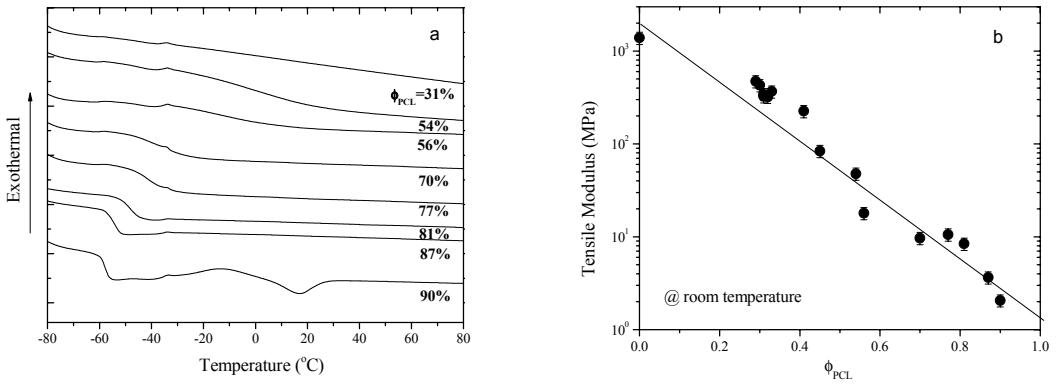
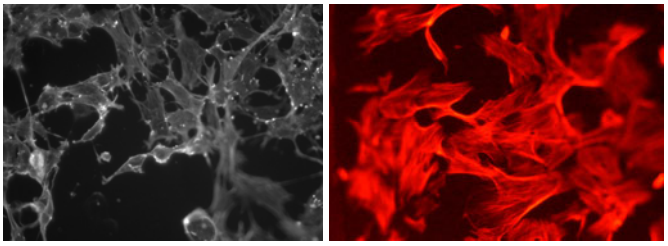


Figure 2. (a) DSC curves and (b) tensile moduli of crosslinked PPF-*co*-PCL samples with different PCL compositions.

Photocrosslinked copolymer disks were first purified by soaking in acetone for 2 days and then dried in vacuum. The disks were sterilized in 80% ethanol and dried overnight in vacuum prior to the cell studies. Cell viability was tested by culturing SPL201 cells at a density of 15,000 per cm^2 and bone marrow stromal cells (BMSCs) at 20,000 per cm^2 in the presence of copolymer disks in transwells at the 24-hr, 4-day, and 7-day conditions. The cell attachment (24 hr) and proliferation (4 and 7 days) were tested by seeding SPL201 cells and BMSCs directly onto copolymer disks. To visualize the morphology by fluorescence microscopy, cells were fixed by paraformaldehyde solution and stained with rhodamine phalloidin and DAPI. To determine the cell number, a colorimetric MTS assay was used. The cell viability of the copolymer disks was found to be close to 100% compared to tissue-culture plate. Fluorescence microscopic pictures in Fig. 3 showed both SPL201 cells and BMSCs attached and proliferated on the copolymer substrates with different PCL compositions of 90% and 30%, respectively. The role of surface rigidity of the polymer substrate on cell responses has been also investigated.

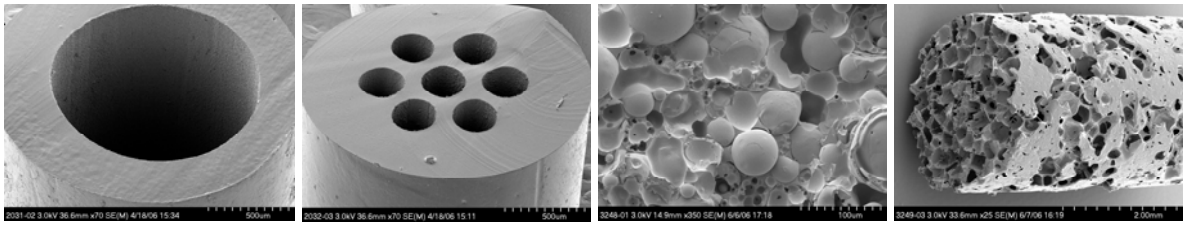
Currently, biodegradable nerve tubes made from polymeric materials are of interest to substitute autologous nerve graft. In this study, one specific PPF-*co*-PCL copolymer with a high PCL composition of 90% was used for the fabrication of single lumen and multi-channel nerve tubes. Similar to the photocrosslinking process described above, the homogenous mixture of PPF-*co*-PCL, BAPO, and CH_2Cl_2 was injected from a syringe to a mold formed by a

glass tube, stainless wires, and Teflon end-caps. Then the mold loaded with polymerizing solution was rotated under UV light for 30 min to facilitate crosslinking. After crosslinking, the formed copolymer nerve tubes were soaked in acetone to wash away the unreacted polymer chains and some other small molecules, and then dried in a vacuum oven. The polymer tubes were sterilized in 80% ethanol for 30 min prior to implantation. The new fabrication method using UV photo-crosslinking proves to be efficient for making tubes without defects (Figs. 4a and 4b). By controlling the degree of crosslinking and impeded crystalline structure, the final copolymer network is strong, which is important for the ability of the nerve to hold the suture, but flexible, which is important in moving body parts.



(a) (b)

Figure 3. (a) SPL201 cells and (b) bone marrow stromal cells on PPF-*co*-PCL disks with PCL compositions of 90% and 30%, respectively, 7 days post seeding.

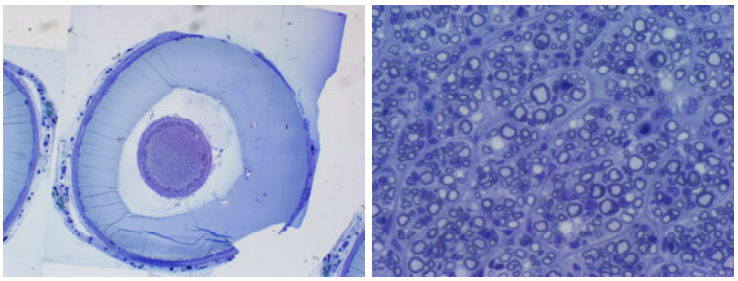


(a) (b) (c) (d)

Figure 4. (a) Single-lumen and (b) multi-channel nerve tubes fabricated from PPF-*co*-PCL with a PCL composition of 90%; (c) Bone implant made from PLGA microspheres containing rhBMP-2 embedded in the matrix of PPF-*co*-PCL with a PCL composition of 30%; (d) A typical bone-tissue-engineering scaffold fabricated from PPF-*co*-PCL with a PCL composition of 30% using salt-leaching technique.

For *in vivo* analysis, polymer tubes were implanted in a 1 cm gap of the rat sciatic nerve model. After 4 and 16 weeks of implantation tubes were removed and imbedded. Sections were taken from the mid-part of the tubes, stained with toluidine blue, and evaluated for the presence of myelinated axons, macrophages, and fibroblasts. Results for *in vivo* implantation showed that the nerve tubes are biocompatible, with only a small layer of fibroblast tissue around the tube. Myelinated axons were present in the mid-part of the channels of the tube 16 weeks after implantation (Fig. 5). The structure of tube was thereby still intact. For bone-tissue-engineering scaffolds, one copolymer with a low PPF composition of 30% was used. Poly(lactide-glycolide) (PLGA) microspheres containing recombinant human bone morphogenetic protein-2 (rhBMP-2) was embedded in the solid polymer rods, as shown in Fig. 4c. Salt-leaching technique was also used to fabricate porous scaffolds with a porosity

of 80% and pore size of 300-400 μm. Rat femoral defect and subcutaneous models were used to test biocompatibility and bone ingrowth with or without the presence of rhBMP-2.



(a) (b)

Figure 5. Section taken through the middle part of a single-lumen nerve tube 16 weeks after implantation at different magnifications: (a) 32×; (b) 800×.

In summary, the crosslinking characteristics and biological evaluations of a novel copolymer, PPF-*co*-PCL, have been investigated. The copolymers with different PCL compositions have different thermal and mechanical properties to satisfy the requirements in bone and nerve regenerations. They also demonstrate high cell viability and support cell attachment and proliferation. Nerve guide conduits and porous scaffolds have been fabricated using this type of biomaterials and excellent biocompatibility has been revealed.

References

1. Wang SF. Macromolecules 2005;38:7358.

Acknowledgments

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