Biological Evaluation of Poly(propylene fumarate)-*co*-Poly(ε-caprolactone) for Bone Tissue Engineering

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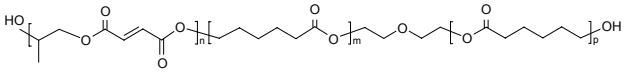
Introduction: One novel copolymer poly(propylene fumarate-*co*-caprolactone) (PPF-*co*-PCL) with 15 compositions of PCL has been invented (Scheme 1) to obtain controllable physical properties for various needs in tissue engineering, particularly, bone and nerve regenerations. The physical properties of the copolymers have been extensively investigated and an important characteristic temperature T_g, i.e., glass transition temperature decreases progressively with increasing the PCL composition in the copolymer. The biodegradation rate and mechanical properties can therefore be well modulated by the different molecular structure, copolymer composition, and crosslinking density. In this study, we demonstrate the biological evaluation of one PPF-*co*-PCL copolymer with a high PPF composition in order to explore its bone tissue engineering applications.



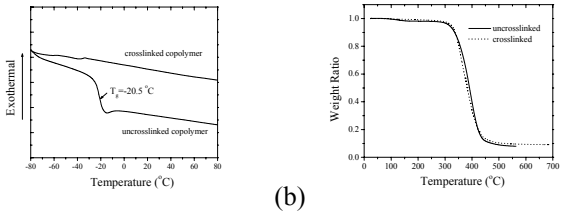
Scheme 1

Methods: PPF-*co*-PCL was produced as described previously.[1] One particular copolymer with a high PPF composition of 70% was used in the present study. The weight-average molecular weight of the copolymer is 25200 g/mol and the number-average molecular weight is 6800 g/mol. Photocrosslinking of the copolymer were initiated with ultraviolet (UV) (λ=315-380 nm) using a photoinitiator bisacylphosphine oxide (BAPO, Ciba Geigy). About 75 µL of BAPO solution (30 mg BAPO in 150 µL CH_2CH_2) was added into 1.5 g PPF-*co*-PCL solution in 500 µL of methylene chloride and mixed thoroughly. The mixture was poured in a mold formed by two glass plates and a Teflon spacer of 0.5 mm thickness and the mold was placed directly under UV light for 30 min to facilitate crosslinking. The gel fraction, swelling ratios in various solvents were determined and the thermal properties were measured by DSC and TGA. The mechanical properties such as compression modulus, flexibility, surface rigidity, and pulling strength have been tested. The copolymer disks were first purified by soaking in methylene chloride and then sterilized in 80% ethanol and dried before the cell studies.

The 4-day cell viability was tested by culturing bone marrow stromal cells (BMSCs) at 20K/cm^2 in the presence of polymer disks in transwells. The cell attachment (24 hr) and proliferation (4 and 7 days) were tested by seeding BMSCs directly on polymer disks. To visualize the morphology by fluorescence microscopy, cells were fixed and stained with rhodamine phalloidin. To determine the cell number, a colorimetric MTS assay was used.

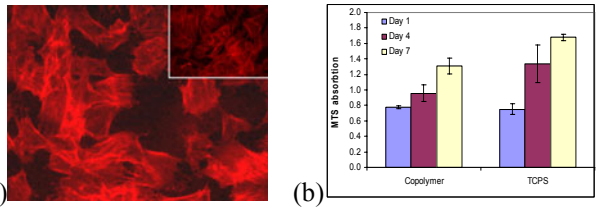
Results/Discussion: The gel fraction of the UV crosslinked copolymer disks is close to 100%. The crosslinked copolymer disks are quite hydrophobic and little swelling can be detected in PBS solution. For PPF-*co*-PCL copolymers at PPF compositions larger than 45%, no discernible glass transition can be found for their photo-crosslinked form due to high crosslinking density. As depicted in Fig.1a, the glass transition around -20.5 ^oC in the uncrosslinked copolymer in this study diminishes after crosslinking. Therefore, the crosslinked copolymer is very rigid and strong to be used as a substitute for PMMA bone cement. Fig.1b shows there is only one thermal degradation step for both copolymer and its crosslinked form. Moreover, the thermal degradation temperature decreases from 346 ^oC for the uncrosslinked copolymer to 332 ^oC for the crosslinked one. The amount of BAPO in the photo-crosslinking also affects the physical properties of the crosslinked copolymer significantly.

The cell viability of the copolymer disks were found to be 96.1±0.8% compared to TCPS control (100±3.5%). Fluorescence microscopy showed cells attached and proliferated on the polymer substrate (Fig. 2a). The quantitative MTS assay showed no difference in cell attachment after 24 hr compared to TCPS. After 4 and 7 days, the proliferation rate on the polymer substrate was slightly less than TCPS.



(a) (b)

Fig.1: (a) DSC and (b) TGA curves of the copolymer and its crosslinked form.



(a) (b)

Fig. 2: (a) MSC attachment on copolymer disk (Inset: TCPS) after 24 hr; (b) proliferation at day 1, 4, and 7.

Conclusions: A novel photo-crosslinkable copolymer PPF-*co*-PCL has been fabricated into disks. It is found that this new material is suitable for orthopedic applications because it has high cell viability and supports cell attachment and proliferation.

References

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

Acknowledgments

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