Reducing Cytotoxicity of Injectable Poly(propylene-co-caprolactone) Copolymers for Bone Tissue Engineering

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Statement of purpose: Biodegradable scaffolds are currently explored in bone tissue engineering strategies aiming to treat severe, critical-sized defects. The scaffolds are expected to provide temporary mechanical stability of the defect site, and as they degrade, the newly formed bone would resume load bearing capability. Our laboratory has synthesized a new copolymer, poly(propylene-co-caprolactone) [P(PF-co-CL)], and have investigated its potential as an orthopaedic biomaterial [1]. The copolymer can form highly cross-linked networks *in situ*, enabling its use as an injectable biomaterial for filling irregularly shaped defects via minimally invasive techniques. The injectable application, however, poses additional challenges for in vivo applications as compared to preformed scaffolds that most research is currently focused on in the biomaterials field. For example, the preformed scaffolds could use photo-crosslinking and could be cleansed by organic solvent (acetone or ethanol) prior to in vivo use. In contrast, injectable scaffolds would require all materials present during *in situ*, chemical crosslinking to be biocompatible. In this study, we systemically evaluated cytotoxicity of chemically crosslinked poly(propylene-co-caprolactone) copolymers without further purification and found ways to improve the biocompatibility of the injectable copolymers.

Methods: **Copolymer synthesis**. P(PF-co-CL) was synthesized using an established three-step methods [2]. Some uncrosslinked copolymer was purified by diethyl ether and 70% ethanol solution for 24 hrs for comparison.

Disk fabrication. The copolymers were crosslinked via radical polymerization with monomers of methyl methacrylate (MMA) or N-vinylpyrrolidinone (NVP), in the presence of benzoyl peroxide (BPO) as initiator and dimethyl-p-toluidine (DMT) as accelerator. Copolymer disks (9×1 mm, diameter×thickness) were crosslinked at 37ºc or 120ºc for 24 h.

Disk characterization. The sol fraction of the copolymer networks was measured after extraction in acetone for 24 h, and calculated as (*W*i - *W*d)/*W*i×100 where Wi and Wd are initial and dried weight, respectively. The extraction solution was analyzed by NMR and FTIR. Thermogravimetric analysis (TGA) was used to determine the weight loss during thermal degradation.

Cell culture and in vitro cytocompatibility. Polymer disks were soaked in culture medium at 37ºC. The medium was changed every 24 h and the extraction medium was collected for up to 7 days. The medium was then added to the cultures of human fetal osteoblast (hFOB) cells. In a separate experiment, sterilized copolymer disks were placed in transwells and added to the cultures of hFOB cells. Cells were exposed to the disks or extraction medium for 72 h. MTS assay was performed to determine cell viability.

Results and Discussion: P(PF-co-CL) copolymer disks were found to be cytotoxic immediately following chemical crosslinking. However, the disks were cytocompatible after extraction in acetone. Analysis of the acetone extracts using NMR and FTIR confirmed the presence of uncrosslinked monomers which were responsible for the cytotoxicity of the chemical crosslinked copolymers. Several methods were then developed to reduce and eliminate such toxic components. First, we further purified the uncrosslinked copolymers using diethyl ether and 70% ethanol for 24 h before crosslinking. TGA results showed that low molecular weight monomers were removed. We then investigated different crosslinking formulations using two crosslinkers (MMA vs. NVP). Sol fraction study showed that P(PF-co-CL) copolymers achieved more complete crosslinking with monomers of MMA (Fig. 1).

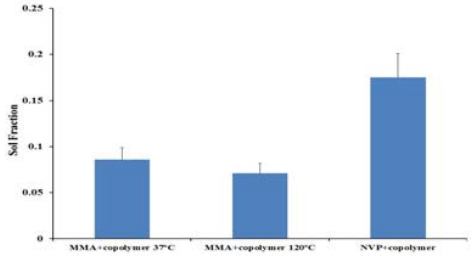

Figure 1. Sol fraction of different copolymer networks.

The cytotoxicity effects of the extracted solution or polymer disks placed in traswells are presented in Fig. 2. Cell viability increased dramatically from 19% after 24 h extraction with acetone and then to 80% after 48 h extraction. Disks rinsed with culture medium for 7 days also exhibited good cell viability (80%).

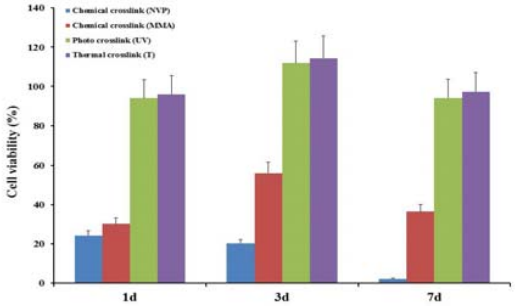

Figure 2. Percent cell viability in the presence of chemically crosslinked polymer disks or extraction medium during 7 days of hFOB cell culture.

Conclusions: We have developed methods to purify the uncrosslinked P(PF-co-CL) copolymer prior to crosslinking. MMA was found to allow more complete crosslinking than NVP. The improved copolymer formulation could potentially be useful as injectable scaffolds for bone tissue engineering.

References 1. Wang S et al. Macromolecules 2005:38:7358-7370.
2. Yan J et al. J Biomater Sci Polym Ed.2011;22:489-504.

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