Biodegradation and Biocompatibility of Photocrosslinkable Oligo(polyethylene glycol) Fumarate Hydrogels: In vitro & In vivo Studies

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Introduction: Oligo(polyethylene glycol) fumarate (OPF) is a novel oligomer that has been developed by Jo et al.[1] OPF can be crosslinked through the unsaturated double bonds in the fumarate groups and hydrolytically degraded through its ester bonds. We have previously demonstrated that OPF can be used for chondrocyte encapsulation[2] and as a scaffold for osteoblastic differentiation of marrow stromal cells. In addition, we have reported that incorporation of a positively charged monomer into the OPF hydrogel has effect on transfection of the encapsulated cells within the hydrogel. In the present study, we describe in vivo and in vitro biodegradation and biocompatibility of the neutral and positively charged OPF hydrogels. In vitro degradation of OPF hydrogel was studied in various biologic-like solutions. In vivo biodegradation and tissue response to the hydrogels were also investigated with subcutaneous implantation of the hydrogel samples into the rat dorsal cavities.

Methods: OPF was synthesized from purified polyethylene glycol with initial MW of 1000 and 10,000 according to a previously published method.[1] Hydrogels were made by dissolving OPF macromer to a final concentration of 33% (w/w) in deionized water containing 0.05% (w/w) of a photoinitiator (Irgacure 2959, Ciba-Specialty Chemicals) and 0.33% (w/w) N-vinyl pyrrolidinone (NVP). In order to obtain positively charged hydrogel, 200 mM [2-(methacryloyloxy) ethyl]-trimethyl ammonium chloride (MAETAC) was added to the solution. The hydrogel mixture was pipetted between the glass slides with a 1 mm spacer and polymerized using UV light (365 nm) at an intensity of ~8mW/cm^2 (Blak-Ray Model 100AP) for 30 min. Hydrogels were prepared as above and placed in the following solutions: PBS, esterase (41 units/mL), and hydrogen peroxide and incubated at 37°C on an orbital shaker. Every three days, the media was replaced with fresh media. At desired time points, media was aspirated and weight loss was measured using gravimetric measurement method.

Hydrogels were cut into the discs with a diameter of 10 mm and swollen in PBS. Hydrogel disks were weighted and disinfected with 70% ethanol, and implanted subcutaneously in the back of 3-month old female Sprague- Dawley rats. Rats were euthanized at 4 and 12 weeks post implantation. Specimens were washed in 1% triton X-100, and rinsed in deionized water followed by drying in low pressure for measurement of weight loss. To characterize the tissue response, the implants with surrounding tissue were harvested and embedded in paraffin using a conventional protocol. Embedded specimens were sectioned and stained with hematoxylin and eosin for light microscope evaluation.

Unpaired t-test was used for statistical analysis of data at the 95% confidence level.

Results: Table 1 shows weight loss of implanted polymers after 4 and 12 weeks post implantation. Overall, similar degradation profile was observed for both hydrogel formulations with and without charge. There was about 10% weight loss for both hydrogels after 4 weeks implantation, which did not change significantly after 12 weeks. Similar trend was shown in our in vitro studies in different solutions including enzyme and PBS. However, progressive degradation of OPF hydrogels in hydrogen peroxide solution was observed.

Table 1

Materials	In Vivo Weight loss (%)	
	4 weeks	12 weeks
Neutral OPF	10.6±1.7	12.0±0.6
Positively Charged OPF	10.1±3.6	6.1±1.0
PLGA	8.3±5.0	32.1±15.5*

Examination of the histological sections of the subcutaneous implants revealed that the tissue response to both OPF formulations was similar to the reaction to PLGA after 4 weeks. A thin fibrous capsule was formed around all samples, which seemed to be thicker after 12 weeks of implantation. The fibrous capsule surrounding the implants mainly consisted of flattened fibroblasts and a few inflammatory cells that were not activated according to the immunostaining with CD68 and CD45 antibody (Figure 2). Our in vitro results from MTS viability assay revealed that the leaching materials from OPF hydrogel did not induce cell toxicity.

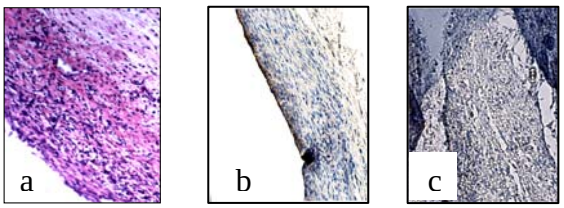

Figure 2: Histological sections of the charged OPF (a) H&E (b) CD45 (c) and CD68 staining after 4 weeks of implantation.

Conclusions: Our results suggest that OPF hydrogel was biocompatible and induced minimal inflammatory response. Incorporation of positively charged monomer into the hydrogel did not affect biocompatibility and biodegradation of hydrogels. Thus, OPF hydrogel has potential for the use in tissue engineering applications in vivo.

Acknowledgment: Work supported by the Mayo Foundation and NIH grants R01 AR45871 and R01 EB003060.

References

1.Jo S et al. Biomacromolecules 2001;2(1):255-61.
2.Dadsetan M et al.Biomacromolecules 2007;8(5):1702-9.