Degradation and Cytocompatibility of a Mixed-Mode Fumarate/Dithiol PEG-based Hydrogel

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Introduction: Our laboratory is investigating mixed-mode oligo[poly(ethylene glycol) fumarate] (OPF)-based hydrogels as cell carriers for regeneration of partial tear ligament defects. The main purpose of this study was to determine the swelling and degradative properties of hydrogels containing three components – OPF, PEG-diacrylate, and PEG-dithiol. In addition, cell viability, morphology changes, proliferation and collagen production from ligament fibroblasts were analyzed in tri-ratio hydrogels with and without the presence of the adhesion ligand Arg-Gly-Asp (RGD) over three weeks of *in vitro* static culture.

Methods: Hydrogel constructs were fabricated from OPF (original poly(ethylene glycol) (PEG) nominal M_n = 10,000 Da), the cross-linker PEG-diacrylate (PEG-DA; M_n = 3400 Da; Laysan Bio, Alabama) and PEG-dithiol (PEG-diSH; M_n = 3400 Da; Laysan Bio) in ratios by weight. The UV photo-initiator Irgacure 2959 (D2959; Ciba, New York) was dissolved in *N*-vinyl pyrrolidone (NVP; Sigma-Aldrich, St. Louis) at a concentration of 0.05% D2959 (weight/total weight) in 10% NVP (weight/polymer weight). The polymer solution was placed in 6 mm diameter by 1 mm thick Teflon molds (~30 µL) and polymerized under UV light (365 nm, 18 mW/cm^2) to create constructs. For degradation studies, wet and dry weights of the hydrogels were recorded over 1 month and fold swelling (wet/dry weight) was calculated (n=3).

For cell encapsulation experiments, hydrogels were fabricated as stated above, RGD was added at 0.01 g/mL and tendon/ligament fibroblasts were encapsulated at 1x10^7 cells/mL. The constructs were cultured in DMEM, containing 10% FBS, 1% non-essential amino acids, 1% HEPES, 0.1% gentamicin, 0.1% fungizone, and 50 µg/mL ascorbate. At timepoints where cell viability was assessed (Days 1, 8, 15 and 20), the constructs (n=3) were stained using LIVE/DEAD dye and imaged on a LSM 510 Confocal Microscope (Zeiss, Germany) for cell viability. At each timepoint, 5 images of each construct were exported to ImageJ for area and circularity analysis. The circularity was calculated from $4\pi A/P^2$, where A and P are the cell area and perimeter, respectively. Other samples were analyzed for DNA content using the PicoGreen assay (Invitrogen, California) and total collagen content using the hydroxyproline assay (n=4). Data from all studies was analyzed using analysis of variance (ANOVA) with Tukey's Multiple Comparison Test ($p \leq 0.05$).

Results and Discussion: The degradation characteristics of PEG-diSH containing hydrogels were compared to non-thiol containing hydrogels (Figure 1). The 60:20:20 OPF/PEG-DA/PEG-diSH (w/w/w) formulation had significantly higher swelling characteristics than the other gel types, and its swelling also increased over time leading to complete degradation by Day 15. While the hydrogel formulation containing 10% PEG-diSH swelled more than the hydrogels containing no PEG-diSH, the fold swelling for both of these gel types remained constant over the timecourse.

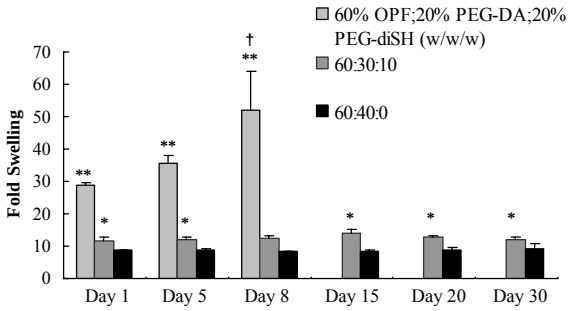

Figure 1. Degradation characteristics of OPF/PEG-DA/PEG-diSH hydrogels. * ** † represent statistical significance relative to (*) 60:40:0 ratios at that timepoint, (**) all other ratios at that timepoint, and (†) Days 1 and 5 for that formulation, respectively.

In terms of effect of gel type on cell morphology (see Figure 2), by Day 8, cell area and circularity analysis showed the area of the cell clusters in both thiol-containing formulations larger than those in the non thiol-containing gels. Cells encapsulated in the hydrogels showed decreased circularity (increased spreading) by Day 8, with the cells in the Thiol + RGD gels demonstrating more elongation compared to cells in both formulations without RGD (Thiol and DA, data not shown).

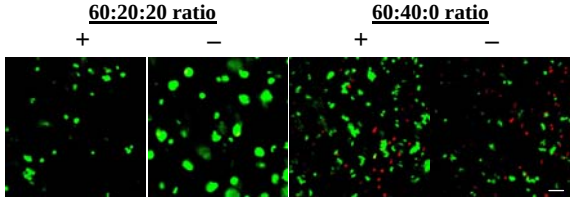

Figure 2. LIVE/DEAD images of ligament fibroblasts in hydrogels with and without PEG-diSH ± RGD at Day 8. (Scale bar = 100 µm)

No trends were seen in DNA content over the timecourse, except a significant decrease in cell number in the DA ± RGD formulations by Days 15 and 20 (data not shown). Total collagen content showed no significant trend over the timecourse (data not shown).

Conclusions: The addition of the PEG-diSH to this system provides an additional means to tune material properties, such as swelling and degradation, of OPF-based hydrogels. The hydrogels resulting from this mixed-mode reaction scheme are cytocompatible and promote cell clustering. Thus, this system holds promise for use in situations where hydrogels are appropriate as cell carriers, such as repair of central defects in tendon/ligament, and other soft tissue regeneration applications.

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