Effect of Inclusion of Microparticle Fillers on Mechanical Properties of PEG-based Hydrogels

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Introduction: Hydrogels have been widely used in biomedical applications due to their ability to exhibit high water contents and tissue-like elastic properties. However, when they are used as tissue-engineering scaffolds in load-bearing applications (i.e. ligament replacement), toughness and modulus of the hydrogel system become vital for effective performance. We are investigating a novel composite microparticle-hydrogel system based on the cytocompatible macromer oligo(poly(ethylene glycol) fumarate) (OPF). In the first part of this study, the loading percentages of physically incorporated microparticles (MPs) [(poly(ethyl methacrylate), calcium carbonate)] were varied to evaluate their effects on the mechanical properties of the composite hydrogel systems. In addition, research has shown that incorporating nanocomposites that are covalently crosslinked with bulk polymer matrices can improve the mechanical properties of the matrix (Biomater. Sci. Polymer Edn.2007;18: p.655). In the second part of this study, to further investigate the effects of microparticle inclusion in a system where the microparticles could be covalently crosslinked to the hydrogel matrix, we have synthesized and characterized a methacrylated alginate system that allows for covalent crosslinking of the alginate in addition to its natural ability to be ionically crosslinked with Ca^{2+} .

Methods: To evaluate the mechanical properties of the composite hydrogel systems, the poly(ethyl methacrylate) (polymer) microparticles [35-45µm, Sigma] and calcium carbonate (ceramic) microparticles [20-30µm, Reade Advanced Materials] were incorporated into 50/50 (wt%) (75% H_2O) OPF/PEG-diacrylate hydrogel at weight percentages of: 0%, 0.5%, 5%, 20%. Hydrogel sheets were then photo-polymerized for 15 min (10mW/cm^2, 365nm, initiator=D2959). OPF3K was synthesized from 3.4 kDa poly(ethylene glycol) (Macromolecules.2001;34:p.2839.). All hydrogels were swelled in saline (PBS) at 37˚C and then die-cut into dogbone samples for mechanical testing using an MTS Insight 2 (Eden Prairie, MN) (n=4 for each treatment). Toughness of the samples was determined by calculating the area under the stress-strain curve, while Young's modulus was measured by calculating the slope of the elastic region. To study the effects of introducing covalent crosslinking in a system that was initially ionically crosslinked, alginate (66% guluronic acid content, 45MPa·s) [Novamatrix, Norway] was methacrylated using carbodiimide chemistry (Biomaterials 2009;30:p.2724). Ionically and covalently crosslinked modified alginate hydrogels (98% H_2O) were fabricated with 650µM concentration of acrylated fluoroscein dye (Sigma), to study dye release from the hydrogel matrices over a 6-day period. Ionically crosslinked alginate hydrogels were made by immersing samples in a 0.7M $CaCl_2$ solution for 10 minutes. Covalently crosslinked hydrogels were made by initial ionic crosslinking per the method described above, followed by covalent crosslinking via photo-polymerization as described above (15 min). All samples (n=4 per treatment) were placed in a 24-well plate with saline solution (PBS) and the supernatant was collected every 2 days and replaced. Supernatant fluorescence counts were analyzed and converted to dye concentration using a SpectraMax M2e microplate reader (Sunnyvale,CA). For statistical analysis, a two-factor ANOVA with Tukey's HSD post hoc test and a Student's t-test was performed on log-transformed data where applicable ($p<0.05$).

Results and Discussion: Tensile measurements revealed that the 20wt% polymer MP samples had a significantly lower modulus than all other sample types, with the exception of the 5wt% polymer MP hydrogels. Similarly, the 20wt% polymer MP gel exhibited significantly lower toughness than all other sample types, except the 0wt% ceramic MP gel (Figure 1). These results indicate that physical incorporation of these MP types at varying loading percentages is not enough to effect a significant change in the toughness of the OPF hydrogel system, and at larger volume fractions the toughness is negatively impacted.

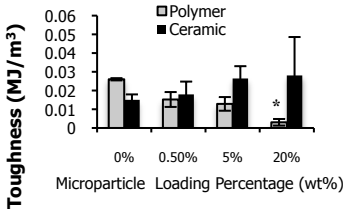

Figure 1. Toughness of OPF/PEG-DA hydrogels with varying loading percentages of polymer and ceramic MPs (n=3). * indicates significantly different from (0,0.5,5% polymer MP gels and (0.5,5,20% ceramic MP gels ($p<0.05$).

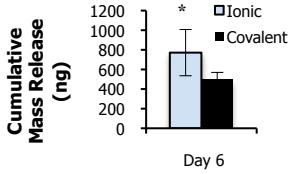

Figure 2. Cumulative mass release of fluoroscein dye (Day 6) from ionically crosslinked and covalently crosslinked alginate hydrogels (n=4). * indicates significantly different from covalently crosslinked gel ($p<0.05$).

After fabrication of the modified alginate, it was observed that the ionically crosslinked gels (ionic) exhibited earlier degradation than covalently crosslinked gels (covalent) (data not shown, ionic gels degraded within 24hrs while covalent gels remained intact up to 4 days), suggesting that the alginate was sucessfully modified and underwent UV-induced crosslinking after ionic gelation. This was further confirmed by the significantly greater release of fluoresecent dye from ionic vs. covalent crosslinked hydrogels by day 6 (Figure 2).

Conclusions: This study demonstrates both the need for, as well as the feasibility of our proposed approach for making microparticles of modified alginate. Such microparticles could be covalently bonded within hydrogel matrices to evaluate their effects on composite toughness, leading to improved hydrogel-based materials for orthopaedic tissue engineering applications.

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