Synthesis and Characterization of Injectable Bioreponsive Hydrogels for Soft Tissue Replacement

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Introduction: An ideal material for the replacement of soft tissue would be one that could be injected through a syringe and solidify within the body, forming to the shape of a defect. Solutions of polymers exhibiting lower critical solution temperatures (LCST) undergo phase transformations from liquid to solid when the temperature is raised above the LCST. Poly(N-isopropylacrylamide) (PNIPAAm), a polymer studied for pharmaceutical and tissue engineering applications[1], typically exhibits an LCST between 32-34°C, making it especially suitable as an injectable implant material. At body temperature however, PNIPAAm is a hydrophobic polymer having a low water content and poor mechanical properties. We have synthesized copolymers of PNIPAAm with poly(ethylene glycol) (PEG) grafts and branches to incorporate additional water into the gel structure with the goal of enhancing the elastic recovery of the gels without the potential biocompatibility risks associated with crosslinking agents[2]. We evaluated the chemistries of a family of injectable polymers and thermal properties of the corresponding aqueous solutions of these polymers. Swelling, dissolution, and mechanical properties of gels formed from aqueous solutions of these polymers were also investigated.

Methods: NIPAAm, 97% (Aldrich) was purified by recrystallization in hexanes at 55°C followed by cooling. Purified NIPAAm was copolymerized with either Poly(ethylene glycol) 1000 monomethyl ether monomethacrylate (PEGM 1000) or Poly(ethylene glycol) 1000 dimethacrylate (PEGDM 1000) with methanol as a solvent at 65°C for 48 hours. The polymerization reactions were initiated with 2,2'-Azobisisobutyronitrile, 98% (AIBN). The ratios of NIPAAm / PEGM 1000 (1/0, 300/1, and 50/1) and NIPAAm / PEGDM 1000 (1/0, 1000/1, 600/1, 300/1, and 50/1) were varied to create a family of injectable polymers. Methanol was evaporated from the reaction mixtures after the polymerizations were complete. The dry polymers were then purified to remove residual monomer by filtration in hot hexanes. The chemistries of the polymers were confirmed with H_1-NMR.

25% aqueous solutions were made from the purified polymers. The LCSTs of the polymer solutions were characterized with DSC. The masses of the gels were recorded between 1 and 90 days of immersion in PBS solution (pH=7.4) at 37°C. The mass swelling coefficient, q(t) was calculated at each immersion point and the water content, dissolution, and polymer chemistries were determined after 90 days immersion. Unconfined compression tests were performed on the hydrogels in PBS at 37°C after 2, 7, and 14 days of immersion in PBS. The compressive modulus was calculated as the slope of each stress/strain response curve.

Results: NMR spectra of this family of grafted and branched polymers were used to verify the addition of PEG grafts and branches to PNIPAAm. The ratios determined by NMR closely matched the intended ratios for each reaction mixture.

Most of the 25% solutions of the grafted and branched polymers had LCST values between 32°C and 35°C, making them suitable for injection at room temperature and precipitation at body temperature. LCST values for the polymer solutions were found to increase as the PEG content was increased. Solutions of grafted and branched polymers with 50/1 NIPAAm/PEGM and 50/1 NIPAAm/PEGDM ratios exhibited LCSTs above 36°C making them unsuitable for injectable soft tissue replacement applications.

The precipitated polymer gels reached an equilibrium swelling level after 2 weeks of immersion in PBS. Swelling of the gels was initially dependent of the concentration of PEG branches. Gels rich with PEG branches (300/1) exhibited significantly higher swelling than gels prepared with less PEG. After 14 days of immersion in PBS, the differences in swelling with PEG concentration were not seen as each of the gels achieved an equilibrium level of swelling (q=1.5–1.7). However, the water content of the gels after 90 days of immersion indicated that the addition of either PEG branches or PEG grafts resulted in an increase in water content confirming that PEG allowed more water to exist within the polymer networks. Pure PNIPAAm gels had an average water content of 35.2% versus 53.9% for 300/1 NIPAAm/PEGDM gels. The 300/1 PEG rich gels suffered from increased polymer dissolution over 90 days of immersion where the pure PNIPAAm, 1000/1, and 600/1 gels all showed less than 3% dissolution. H_1-NMR confirmed that there were no changes in polymer chemistry for all of the gels as a result of 90 days immersion.

Gels prepared with PEG branches initially exhibited higher mass swelling coefficients than gels prepared with PEG grafts, indicating they were better able to entrap water within their networks (Figure 1). These differences however, were negligible after 14 days of immersion. The initial differences between PNIPAAm with PEG branches and PEG grafts show that branches are more effective in forming networks capable of holding greater amounts of water.

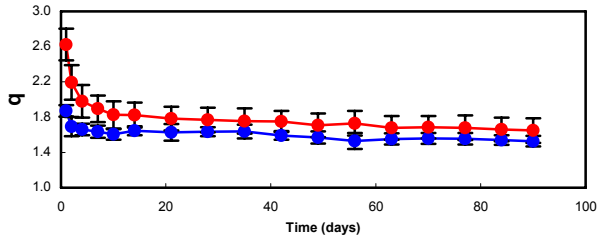

Figure 1. Mass swelling coefficients of gels prepared with NIPAAm/PEG ratio of 300/1 with PEG grafts (●) and branches (●).

The compressive modulus values for this family of polymers ranged from 20 kPa to 100 kPa. The modulus of pure PNIPAAm gels did not change with immersion time, however gels prepared with 600/1 and 300/1 NIPAAm/PEGDM stiffened with immersion. This stiffening was attributed to loss of water between 2 days and 14 days of immersion. Gels prepared with 600/1 NIPAAm/PEGDM exhibited higher modulus values than pure PNIPAAm and 300/1 NIPAAm/PEGDM. Gels prepared with 300/1 NIPAAm/PEGDM exhibited significantly better elastic and dimensional recovery over pure PNIPAAm gels.

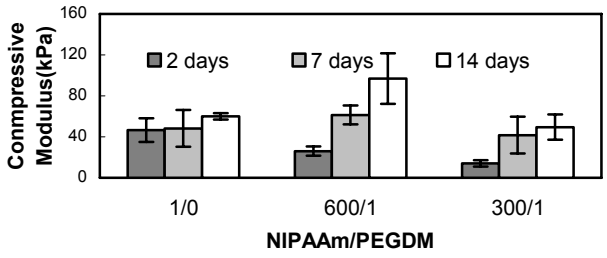

Figure 2. Compressive modulus values for PNIPAAm with PEG branches after 2, 7, and 14 days of immersion in PBS.

Conclusions: We have observed the ability of moderate amounts of PEG branches and grafts within PNIPAAm to raise the equilibrium water content of the precipitated polymers without drastically changing the LCST of the polymer. Higher concentrations of PEG grafts or branches yielded a network of PEG that when placed in solution, increased the LCST beyond one ideal for clinical application. Polymerization with a range of PEG concentrations resulted in compressive properties and elastic behavior depending on the concentration of PEG grafts or branches and the stability over time of immersion *in vitro*. This material system offers tunable mechanical properties with mass stability in an injectable, phase transforming materials which may be suitable for a variety of soft tissue applications.

References:

1. RA Stile and KE Healy. Biomacromolecules, 2002, 3, 591-600.
2. AM Lowman, MS Marcolongo, and A Clemow. Patent Application No.60/466,819.

Acknowledgements: Synthes-Spine for funding this study.