Simultaneously Physically and Chemically Crosslinking Thiol Functionalized N-Isopropylacrylamide with Poly(ethylene glycol) diacrylate for Functional Embolization

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Statement of Purpose: Poly(N-Isopropylacrylamide) physical gels have previously been investigated for use in functional embolization (Vernon BL J Biomater Sci Polym Ed. 2005; 16:1153-1166). Experiments revealed that, while sufficiently strong for implantation, purely physical gels suffered from long term creep under constant stress. Because of this limitation, new systems have been designed which cure over time to eliminate creep and augment the initial physical gelation.

Methods: Materials. N-Isopropylacrylamide (NIPAAm) was purified by recrystallization in hexanes and dried under vacuum for four days. 2,2'-Azobisisobutylronitrile (AIBN) was purified by recrystallization in methanol. N-acryloxysuccinimide (NASI), poly(ethylene glycol) diacrylate (PEGDA) MW 700, cysteamine hydrochloride, triethylamine (TEA), and isopropylamine were used as received. Anhydrous 1,4-dioxane, dimethylchloride (MC) and all other solvents used in this experiment were used as received.

Methods. NIPAAm based copolymers with NIPAAm/NASI 98:2 were synthesized by radical polymerization in dioxane, as in the literature (Yang HJ. J Poly Sci 1990; 28:219-226). Once dissolved, AIBN was added and the reaction proceeded for 24 h at 65^{o}C. The polymer solution was precipitated in an excess of diethyl ether, filtered and vacuumed dried. Ten grams of poly(NIPAAm-co-NASI) and 0.295 g of cysteamine hydrochloride were dried separately for 24 h under vacuum at 60^{o}C to reduce moisture and then dissolved in MC. TEA was added to the cysteamine hydrochloride solution and stirred until dissolved. Once dissolved, the solutions were combined and stirred at room temperature for 24 h. The polymer solution was precipitated in an excess of diethyl ether, filtered, vacuumed dried and stored in a cool environment until further use. Cysteamine content was determined using H-NMR and Ellman's Method (Ellman GL. Arch Biochem and Biophys 1958; 74:443-450). Light spectroscopy was used to determine the pKa of NIPAAm-co-Cysteamine (Lutolf MP. Bioconj Chem 2001; 12:1051-1056). Remaining thiol content during gelation as a function of pH and amount of PEGDA was determined using Ellman's Method. The storage modulus (G') and gelation kinetics were investigated using rheometry.

Results/Discussion:

Ellman's Method showed 1.99% thiol content (1.757 x 10^{-4} mol thiol/g polymer). The pKa of the polymer was determined to be 8.85. This pKa was expected, as the pKa of cysteamine alone is 8.35 and attachment to a polymer backbone is expected to raise the pKa slightly (Danehy JP. J Chem and Eng Data 1968; 13:386-389). A kinetic study was performed to show how thiol content varies with pH and with the amount of PEGDA. When there is no PEGDA present, gelation is solely based on disulfide bond formation and occurs over hours to days. Figure 1 shows that when there are equal amounts of thiol and acrylate, gelation occurs more rapidly due to Michael Type Addition Reaction. Additionally, gelation occurs more rapidly at higher pH (closer to the pKa) because thiolate ions form more easily. When acrylate concentration is increased further (10X amount of thiol), gelation occurs even more rapidly due to the increased availability of acrylates resulting in faster reaction times (data not shown).

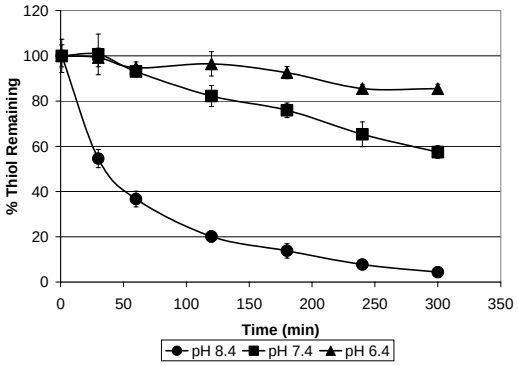

Figure 1. Time dependent thiol concentration with a thiol:acrylate concentration of 1:1.

Physical gelation of NIPAAm-Cysteamine occurs at 26 ^{o}C, as shown in Figure 2A. Physical gelation occurs due to hydrophobic interactions between the methacrylate groups on the NIPAAm. Chemical gelation of NIPAAm-Cysteamine with PEGDA, shown in Figure 2B, occurs almost instantaneously at equal thiol to acrylate concentrations.

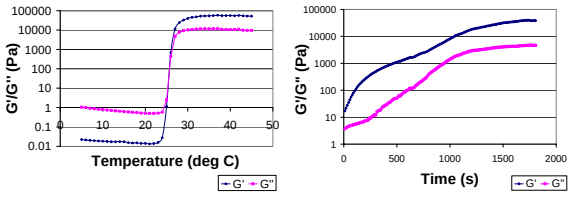

Figure 2. (A) Temperature sweep of NIPAAm-Cysteamine showing physical gelation. (B) Time sweep of NIPAAm-Cysteamine with PEGDA showing chemical gelation. Both traces are at pH 7.4.

Comparison of the traces shows that the material retains its temperature sensitivity, but is also capable of providing a chemical gel over time at the appropriate pH.

Conclusions: This work suggests that a combination of physical and chemical gelation results in high strength, low creep materials optimal for functional embolization.

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