Simultaneously Physically and Chemically Gelling Polymer System Utilizing a Poly(NIPAAm-*co*-cysteamine)-Based Copolymer

Stephanie A. Robb, Bae Hoon Lee, Ryan McLemore, and Brent L. Vernon*

The Harrington Department of Bioengineering, Center for Interventional Biomaterials, ECG 334, Arizona State University, Tempe, Arizona 85287-9709

Received March 6, 2007; Revised Manuscript Received May 1, 2007

The objective of this work was to create an in situ physically and chemically cross-linking hydrogel for in vivo applications. *N*-Isopropylacrylamide (NIPAAm) was copolymerized with *N*-acryloxysuccinimide (NASI) via free radical polymerization. Poly(NIPAAm-*co*-NASI) was further modified to obtain poly(NIPAAm-*co*-cysteamine) through a nucleophilic attack on the carbonyl group of the NASI by the amine group of the cysteamine. Modification was verified by nuclear magnetic resonance. In addition to thermoresponsive physical gelling due to the presence of NIPAAm, this system also chemically gels via a Michael-type addition reaction when mixed with poly(ethylene glycol) diacrylate. The presence of both physical and chemical gelation resulted in material properties that are much improved compared to purely physical gels. The chemical gelation time of the copolymers was not significantly affected by the amount of thiol present due to the increased pK_a of the copolymer containing more thiols. In addition, the swelling of the copolymers was highly dependent on the temperature and thiol content. Last, the rate of nucleophilic attack in the Michael-type addition reaction was shown to be highly dependent on pH and on the mole ratio of thiol to acrylate. Due to the improved mechanical properties, this material may be better suited for long-term functional replacement applications than other thermosensitive physical gels. With further development and biocompatibility testing, this material could potentially be applied as a temperature-responsive injectable biomaterial for functional embolization.

Introduction

Injectable, in situ-forming biomaterials have recently become highly desirable due to their numerous unique advantages, including increased ease of use and the minimally invasive procedures used for their site specific introduction into the body relative to traditional surgical techniques.[1] Additionally, for applications where the final shape of the material is defined by the local in vivo environment, such in situ-forming biomaterials are ideal.[2,3]

Intervenient embolism therapy is currently considered one of the main treatment methods for arteriovenous malformations (AVMs)[4,5] and aneurysms. Biomaterials used for occlusion include cyanoacrylate derivatives, polyvinyl alcohol particles, gel spheres, and precipitant gels.[2,4] Several hazards exist with the use of such embolic materials, however. Complications can include escape of the material, resulting in nontreatment and possible formation of microemboli, leading to stroke, local vascular damage, and adhesion of the application catheter within the cerebral vessel.[2,4,6] In addition, many occlusion biomaterials require organic solvents such as dimethylformamide (DMF), ethyl alcohol, and *N*-methylpyrrolidone (NMP) for delivery, causing solvent toxicity.[4,7] Thus, a water-based polymer system is highly desirable. However, water-based systems generally have excessive swelling and low strength, making them impractical for these applications.

Temperature-responsive sol−gel systems have been investigated for several potential biomedical and pharmaceutical applications.[7−10] Such materials undergo abrupt changes in solubility in response to temperature changes in aqueous solutions. Through increases in the environmental temperature, gelation occurs. These temperature-sensitive materials exhibit viscoelastic flow under constant, low-frequency stress.[7,11] This property can be used for embolization of neurological AVMs, as the material has to be delivered via a catheter that is introduced to the femoral artery in the leg. The catheter is maneuvered through extensive vasculature in the body and into the AVM in the brain. Therefore, the material must remain viscous throughout the delivery process. However, materials that exhibit viscoelastic flow under constant, low-frequency stress can creep due to low-pressure constant stress present due to blood flow. Higher mechanical properties, including low-frequency load bearing, are required in materials designed for functional embolization to prevent creep of the material under long-term, low-magnitude constant stresses in the vascular system. Additional chemical curing after thermal gelation can be used to reduce the viscoelasticity of these materials after delivery. Conjugate addition reactions between thiols and acrylates (also termed Michael-type addition reactions) are currently investigated as such a chemical curing method.[12−14] This reaction can be carried out at physiological temperature and pH, with minimal competition from naturally occurring amines and other nucleophiles.[12,13]

Our group has investigated poly(ethylene glycol) diacrylate (PEGDA)/QT and PPODA/QT as in situ-gelling materials that form embolization materials through Michael-type addition reactions. Although these materials showed promise, high pH is required to make QT soluble, the kinetics of QT are very difficult to control, and emulsion can occur.[10] QT has also been mixed with poly(NIPAAm-*co*-HEMA-acrylate) (NIPAAm is *N*-isopropylacrylamide) to form in situ-gelling materials for

* To whom correspondence should be addressed. Tel: 1-480-965-0929. Fax: 1-480-727-7624. E-mail: Brent.Vernon@asu.edu.

10.1021/bm070267r CCC: $37.00
Published on Web 06/13/2007

endovascular embolization.[11] Due to the presence of NIPAAm, the QT/poly(NIPAAm-*co*-HEMA-acrylate) system could gel even in a pH 7.4 solution, eliminating the need for a basic catalyst. This system utilized both physical cross-linking due temperature-responsive NIPAAm and chemical cross-linking via Michael-type addition reactions between the acrylate and QT. Although this system showed increased strength and increased low-frequency load bearing, QT is a very toxic monomer that, if leached from the material, can be quite toxic.

When dissolved in water, the novel material created herein can be mixed with PEGDA at room temperature and injected in vivo. Gelation will occur immediately due to temperature-driven physical cross-linking in the body. The physically cross-linked gel will then cure by chemical cross-linking through Michael-type addition reactions between thiols and acrylates. This tandem system should result in a temperature-dependent, chemical gel that displays high strength and increased low-frequency load bearing.

Therefore, in the present study, a temperature-sensitive copolymer of NIPAAm individually functionalized with thiols was synthesized and characterized to evaluate the feasibility of developing a system that can simultaneously physically and chemically gel.

Materials and Methods

Materials. *N*-Isopropylacrylamide (NIPAAm, Spectrum) was purified by recrystallization in hexanes and dried under vacuum for 4 days. 2,2′-Azobisisobutylronitrile (AIBN, Aldrich) was purified by recrystallization in methanol. *N*-Acryloxysuccinimide (NASI, Aldrich), poly(ethylene glycol) diacrylate (PEGDA, Aldrich, MW 575), cysteamine hydrochloride (Spectrum), triethylamine (TEA, Aldrich), dithiothreitol (DTT, Aldrich), and isopropylamine (Aldrich) were used as received. Anhydrous 1,4-dioxane (Aldrich), methylene chloride (MC, Aldrich), and all other solvents used in this experiment were used as received.

Synthesis of Poly(NIPAAm-*co*-NASI) and Poly(NIPAAm-*co*-cysteamine). NIPAAm-based copolymers with NIPAAm/NASI at the feed ratios of 98:2 and 92:8 were synthesized by radical polymerization in dioxane, as in the literature.[15] Briefly, NIPAAm and NASI were dissolved in dioxane (10 wt %) in a deoxygenated polymerization flask via an alternating connection to vacuum and nitrogen. AIBN was added after 15 min of nitrogen purging and the reaction proceeded for 24 h at 65 °C. The polymer solution was precipitated in an excess of diethyl ether, filtered, and vacuum-dried. Ten grams of poly(NIPAAm-*co*-NASI) and varying amounts of cysteamine hydrochloride (depending on the required mole percent) were dried separately for 24 h under vacuum at 60 °C to reduce moisture and then dissolved in MC. TEA was added to the cysteamine hydrochloride solution to deprotect hydrochloride, and both solutions were 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, and vacuum-dried. The resulting polymers were dissolved, and DTT (equal mole ratio to cysteamine hydrochloride) was added to reduce disulfide bonding. The solutions were then dialyzed (3000 MWCO) at 5 °C against 5 mM HCl, two times against 5 mM HCl containing 1% NaCl, and three times against 5 mM HCl. Extensive dialysis was required to fully remove DTT from the polymer solution. Finally, the aqueous polymer solutions were lyophilized and stored in a cool environment until further use.

^{1}H Nuclear Magnetic Resonance Spectroscopy (H NMR). H NMR measurements were made with a Varian Gemini-300 spectrometer operating at 300 MHz in the Fourier-transform mode. $CDCl_3$ and D_2O were used as the solvent.

Differential Scanning Calorimetry (DSC). The lower critical solution temperature (LCST) of each copolymer was determined using a CSC 4100 multicell differential scanning calorimeter. Scans were performed on 5 wt % polymer solutions in 0.1 M phosphate buffer solution (PBS) of pH 7.4 at 1 °C/min from 0 to 80 °C. Each sample was run in triplicate.

Free Thiol Determination. The free thiol content of each polymer was determined using Ellman's method.[16] Briefly, polymer samples were dissolved at 1 mg/mL in 0.1 M PBS pH 7.4 and mixed with a 10 mM DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)] solution. The absorbance ($n = 4$) at 415 nm was taken using a Bio-Rad Benchmark microplate reader. The thiol content was determined via a cysteine calibration curve of thiol content vs absorbance.

Molecular Weight. The molecular weight of each copolymer was determined using static light scattering (Wyatt Minidawn, Santa Barbara CA) in conjunction with gel permeation chromatography (GPC). GPC (Shimadzu, micro-styragel HR-5 column) was performed on the copolymers with THF as mobile phase and a flow rate of 0.8 mL/min.

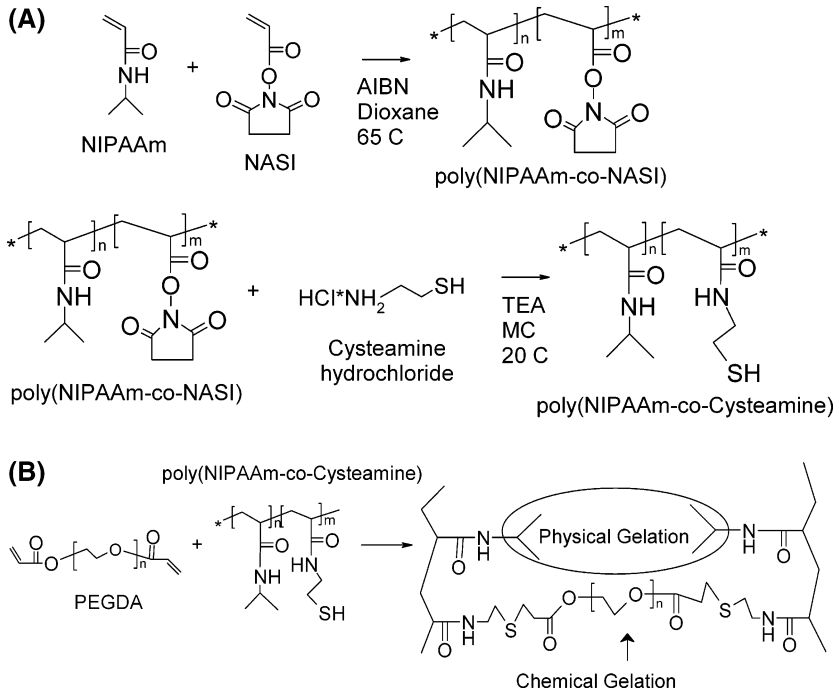
pK_a Determination. The pK_a of thiols in poly(NIPAAm-*co*-cysteamine) was determined spectrophotometrically on the basis of the UV absorption of thiolates.[17] The absorption at 233 nm was measured for each poly(NIPAAm-*co*-cysteamine) copolymer at various pH having identical concentrations using a Pharmacia Biotech Ultrospec 3000 spectrophotometer. Polymer solutions were prepared in a two-component buffer system depending on pH, as described in the literature.[17] Briefly, the two-component system included a phosphate (pH 4−8) or sodium biocarbonate (pH 9−12.5) buffer and 0.1 M NaCl (stable ionic strength). Absorptions at 233 nm of freshly prepared solutions were immediately measured ($n = 3$). The absorption data at 233 nm were plotted as $-\log[(A_{max} - A_i)/A_i]$ vs pH, where A_{max} is the maximum absorbance recorded and A_i is the absorbance at the specified pH. The pK_a value corresponds to the intercept with the *x*-axis.

Kinetic Study. Remaining thiol content during gelation, as a function of pH and amount of PEGDA, was determined using Ellman's method,[16] as described previously.[17,18] Briefly, polymer samples were dissolved at 1 mg/mL in 0.1M PBS of pH 6.4, 7.4, and 8.4 (three samples at each pH were dissolved). After dissolution, PEGDA was added at a ratio of 1:1 and 10:1 (acrylate:thiol). No acrylate was added to the third sample. Absorption readings ($n = 4$) at 415 nm were taken using a Bio-Rad Benchmark microplate reader at times of 1 min, 30 min, 1 h, 2 h, 3 h, 4 h, and 5 h. Results were recorded as percent of thiol remaining vs time.

Gelation Properties. Quantification of the elastic or storage modulus and the gelation kinetics were investigated using a TA Instruments AR1000 rheometer. A 20 mm cone and plate geometry (1.59° down) with a 50 μm truncation was used for each run. All polymer samples were dissolved at a concentration of 15 wt % in 0.1 M PBS pH 5.4 under nitrogen. Nitrogen purging and low pH were used to minimize disulfide bonding during dissolution. To measure temperature-dependent physical gelation, temperature sweeps were performed from 5 to 45 °C with a heating rate of 2 °C/min. The data was collected at a frequency of 1 Hz and a controlled stress of 10 Pa (linear viscoelastic region). The sol-to-gel transition temperature was defined as the temperature at which the storage modulus (G') is equal to the loss modulus (G''). To measure chemical gelation, polymer samples were mixed stiochiometrically with PEGDA (MW 575) for 10 s via syringes. After mixing, 70 μL of the polymer sample was placed on the rheometer and time sweeps were performed at 20 °C for 30 min. The data were collected at the frequency and stress specified above. After gelation, frequency sweeps were performed on the poly(NIPAAm-*co*-cysteamine) with PEGDA samples. Frequency sweeps were performed from 0.01 to 100 Hz at 20 and 37 °C with a controlled stress of 10 Pa. For the 37 °C frequency sweeps, water was absorbed off of the polymer gel prior to running the sweep, and a 40 μm truncation was used (required due to shrinking). Frequency sweeps were also performed on purely physical gels (no acrylate) from 0.01 to 100 Hz at 37 °C with a controlled stress of 10 Pa.

Swelling Test. Samples of poly(NIPAAm-*co*-cysteamine) (15 wt %) with various thiol content (2% or 8% thiol) were dissolved at pH 7.4 and subsequently mixed stoichiometrically with PEGDA via

Scheme 1. (A) Synthesis Scheme of Poly(NIPAAm-*co*-NASI) and Poly(NIPAAm-*co*-cysteamine) and (B) Physical and Chemical Gel Formation of Poly(NIPAAm-*co*-cysteamine) with PEGDA.



syringes for 1 min. After mixing, the sample was incubated at 37 °C for 24 h for further curing. A cylindrical-shaped gel was extracted from the syringe, cut into smaller samples, and lyophilized. The dried gel samples were weighed and then incubated in 0.1 M PBS at pH 7.4 at temperatures of 37, 20, and 5 °C. Samples ($n = 3$ for each polymer) were weighed at specified time intervals of 30 min and 1, 2, 4, 6, 12, 24, and 48 h. The degree of swelling is defined as $[100(W - W_0)/W_0]$, where W is the weight of the swollen gel and W_0 is the weight of the dried polymer.

Statistical Analysis. The means and standard deviations of LCST, pK_a, gelation time, and swelling for both copolymers were compared using a two-sided *t*-test. The level of significance was set to $p < 0.05$.

Results and Discussion

Poly(NIPAAm-*co*-NASI) was prepared by free radical polymerization, as shown in Scheme 1A (top). Conjugation of NASI was verified by ^{1}H NMR, as shown in Figure 1 (top). Poly(NIPAAm-*co*-cysteamine) was synthesized through a nucleophilic attack on the carbonyl group of the NASI by the amine group of the cysteamine (Scheme 1A, bottom). The mole ratio of NIPAAm and cysteamine was calculated from the integration ratio between the methyl protons [6H, $(CH_3)_2$-CHNHCO] of the NIPAAm and the methylene protons (4H, NHCH_2CH_2SH) of cysteamine appearing at 3.3 and 2.6, respectively, as shown in Figure 1. The thiol composition of copolymer 2 determined by NMR may be lower than the actual thiol composition, due to the difficulty of reading such small peak sizes. The mole ratio of cysteamine was also found using Ellman's method.[16] The composition of each copolymer can be found in Table 1. Chemical gelation between the copolymer and PEGDA, via a Michael-type addition reaction, is shown in Scheme 1B.

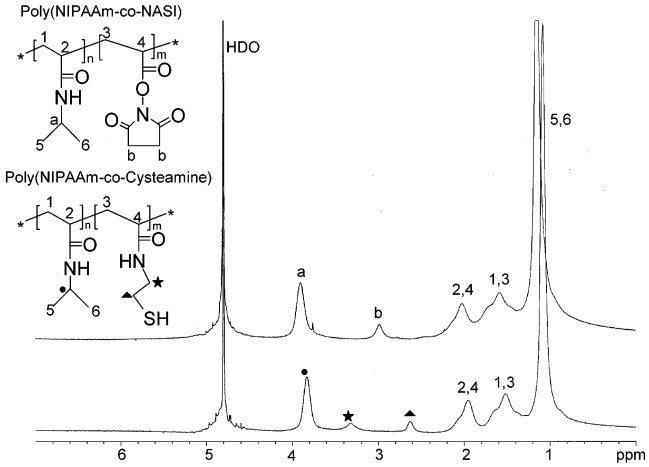


Figure 1. ^{1}H NMR spectra of poly(NIPAAm-*co*-NASI) and poly(NIPAAm-*co*-cysteamine).

The rheology gelation temperature and LCST of copolymers 1 and 2 can be seen in Table 1. The gel temperature of both poly(NIPAAm-*co*-cysteamine) copolymers was determined by rheology to be 26 °C at 15 wt % PBS solutions of pH 5.4 (Figure 2A). A pH of 5.4 was used to slow the formation of disulfide bonding during the dissolution and measurement. The temperature-driven gelation of both copolymers is attributed to the presence of the hydrophobic methyl groups on the NIPAAm. The storage modulus (G') of copolymer 1 is lower than that of copolymer 2 due to increased shrinking at temperatures above the LCST. Shrinking may be more prevalent in copolymer 1 than in copolymer 2 due to more disulfide bond formation during dissolution, and shrinking results in similar values for the storage modulus and the loss modulus (G''), as seen in Figure 2A. As previously reported, the LCST of poly(NIPAAm) is 30 °C when dissolved in 0.1 M PBS, but addition of comonomers to NIPAAm can result in large deviations from this value.[19] The LCST of both copolymers was determined using differential scanning calorimetry with the LCST reported as the peak temperature. The LCST of copolymer 1 was determined to be 29.7 ± 0.10, while the LCST of copolymer 2 was determined to be 29.3 ± 0.14 (Figure 2B) ($p > 0.05$). From this data, it is apparent that the cysteamine has a small significant impact on the LCST of the NIPAAm copolymers. As shown in Figure 2B, the molar heat capacity of copolymer 1 is less than that of

Table 1. Thiol Content, Gelation Temperature, and Molecular Weight of Each Copolymer

	feed ratio		thiol content (10^{-4} mol thiol/g polymer)		transition temperature (°C)			
name	NIPAAm	cysteamine	NMR	Ellman method	DSC peak	rheology ($\Delta = 45°$)	MW ($\times 10^{-5}$ g/mol)	PDI
copolymer 1	92	8	8.334	9.627	29.7 ± 0.10	26	1.04	2.14
copolymer 2	98	2	0.45	1.757	29.3 ± 0.14	26	1.11	1.94

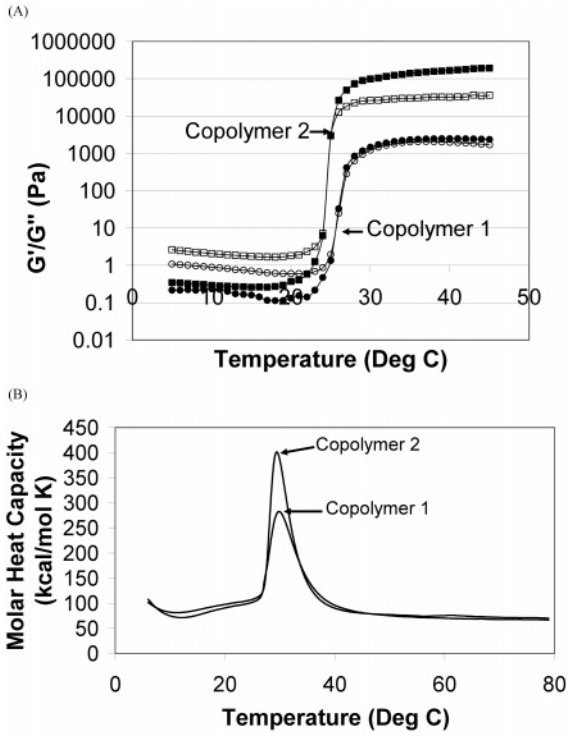


Figure 2. (A) Temperature sweeps showing G' and G'' for each copolymer [copolymer 1, G' (●) and G'' (○); copolymer 2, G' (■) and G'' (□)]. (B) DSC scans indicating the transition of each copolymer.

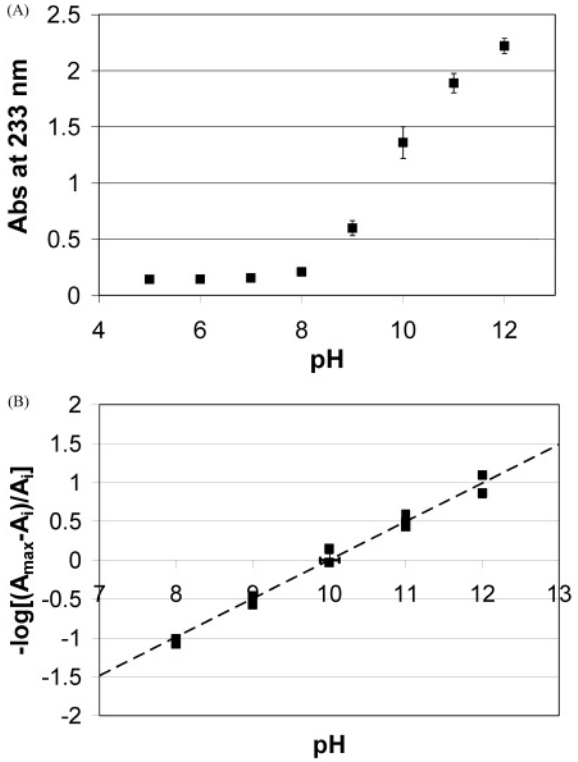


Figure 3. (A) Absorbance at 233 nm as a function of the pH for copolymer 1 ($n = 3$). (B) $-\log[(A_{max} - A_i)/A_i]$ vs pH, where the pK_a value corresponds to the intercept with the y-axis. The linear approximation was only used in the buffer region, as indicated by the dotted line (B).[17]

copolymer 2. This results because, as the mole percent of cysteamine is increased, less NIPAAm groups undergo collapse (phase transition) at the LCST; thus less energy is needed to break the hydrogen bond between water and NIPAAm.[20] The presence of cysteamine groups hinders the complete collapse of the NIPAAm chains. The molecular weight of each copolymer was determined using gel permeation chromatography. As shown in Table 1, copolymer 1 had a number average molecular weight of 4.85×10^4 g/mol, a weight average molecular weight of 1.04×10^5 g/mol, and a polydispersity index (PDI) of 2.14, while copolymer 2 had a number average molecular weight of 5.72×10^4 g/mol, a weight average molecular weight of 1.11×10^5 g/mol, and a PDI of 1.94.

The pK_a of both copolymers was determined spectrophotometrically at 233 nm. The titration curve for copolymer 1 is shown in Figure 3A. At high pH, thiols become completely dissociated, resulting in a maximum absorbance (A_{max}). As reported previously, the intercept with the abscissa in a graph of $-\log[(A_{max} - A_i)/A_i]$ vs pH yields the pK_a value (Figure 3B).[17] The pK_a of both copolymers was determined by fitting a line to the points in the linear region (pH 8−12) and finding the x-intercept of the fit. The pK_a of copolymer 1 was determined to be 10.0 ± 0.12, while the pK_a of copolymer 2 was 9.1 ± 0.26 ($p < 0.05$). The pK_a of cysteamine alone is 8.05−8.35.[21] The increase in pK_a in copolymer 2 (9.11 ± 0.26) and copolymer 1 (10.00 ± 0.12) relative to cysteamine occurs because, as thiolates are created, subsequent thiolate creation constrained near already existing thiolates is hindered by electrostatic repulsion.

In order to observe the effects of pH and mole ratio of thiol to acrylate on the Michael-type addition reaction between each copolymer and PEGDA, a kinetic study was performed. Figure 4 shows the results for copolymer 1. As shown in Figure 4A, disulfide bond formation (i.e., no PEGDA present) is very slow, even at higher pH. The thiol remains stable for 5 h or more at a pH of 7.4 or lower. At a pH closer to the pK_a (pH 8.4), thiolate formation, and therefore disulfide bonding, occurs more quickly with about 70% of the thiols remaining after 5 h. Samples synthesized without DTT during dialysis showed considerably less stability, however. The Michael-type addition reaction between copolymer 1 and PEGDA with a ratio of thiol to acrylate equal to 1:1 results in a quick decrease in the amount of thiol (Figure 4B). At pH 6.4, 75% of the thiol remained after 5 h, while at pH 8.4 all of the thiol had been consumed through reaction. The addition reaction between the copolymer and PEGDA consumes thiols faster than the reaction between thiol radicals. Disulfide bonding requires the presence of oxygen to promote free radical transfer of an electron from a thiolate, forming a thiol radical. Thiol radical formation must then be followed by a weak electrophilic attack. Michael-type addition reactions occur quickly relative to disulfide bonding because

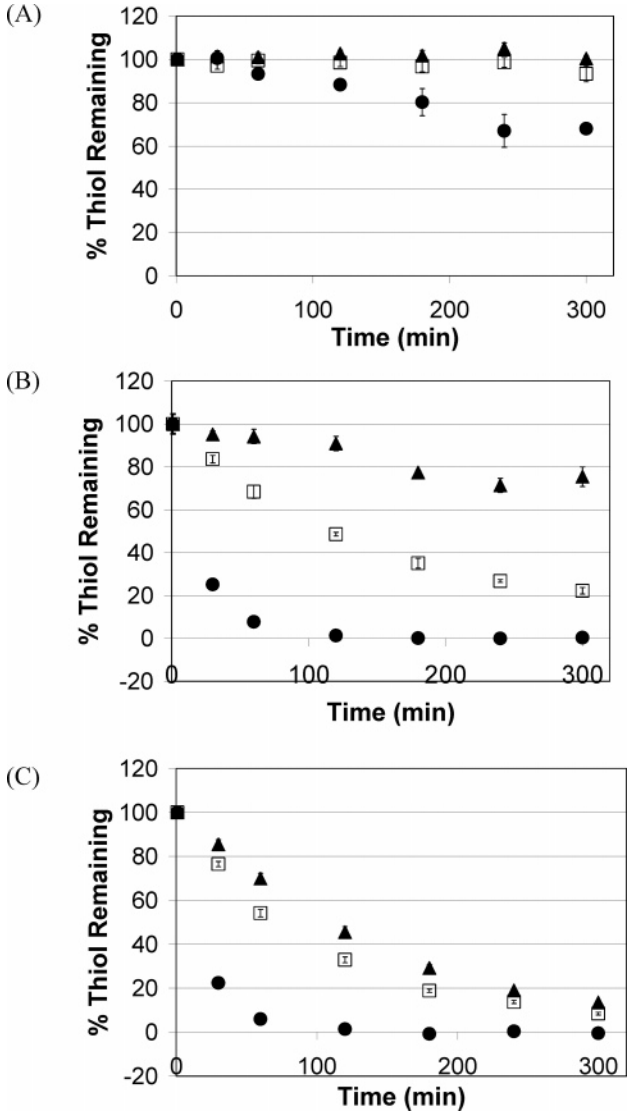


Figure 4. (A) Kinetic study of disulfide bond formation for copolymer 1 at various pH (pH 6.4, ▲; pH 7.4, □; pH 8.4, ●). (B) Kinetic study of the Michael-type addition reaction between thiols and acrylates (ratio 1:1) for copolymer 1 at various pH. (C) Kinetic study of the Michael-type addition reaction between thiols and acrylates (ratio 1:10) for copolymer 1 at various pH. ($n = 4$ for all).

thiolates are strong nucleophiles, initiating faster reactions than the weak electrophilic combination of thiol radicals. As thiolates are formed, they are permanently consumed by a reaction with an acrylate. To maintain a constant thiol to thiolate ratio in solution (as determined by the pK_a) during this consumption, more thiols become thiolate, which in turn react with acrylate. At higher pH, the ratio of thiolates to thiols is greater, resulting in faster elimination of thiols. As the amount of PEGDA is increased to a thiol to acrylate ratio of 1:10, the Michael-type addition reaction occurs even faster (Figure 4C). This occurs because when more acrylates are available for reaction, the second-order rate dynamics result in an increased thiolate consumption per unit time. The data for copolymer 2 showed similar trends (data provided as Supporting Information).

The time dependence of the dynamic moduli for the chemical gelation of both copolymers is shown in Figure 5. All samples were dissolved at 15 wt % in pH 5.4 PBS to avoid disulfide bonding (disulfide bonding becomes more prevalent at higher weight percentages due to increased local concentrations of thiol radicals). The polymer samples were stoichiometrically mixed

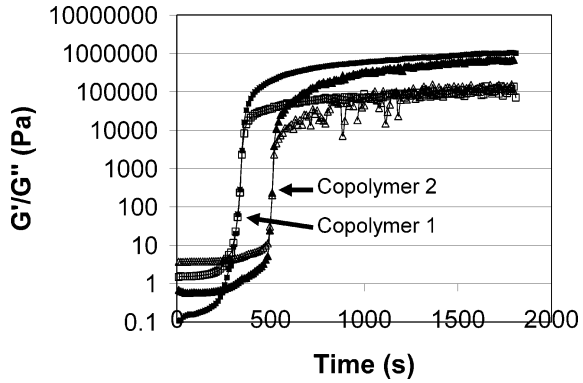


Figure 5. Time sweeps of copolymer 1 [G' (■) and G'' (□)] and copolymer 2 [G' (▲) and G'' (△)]. The gel point for each is indicated by an arrow, and is defined as the point where G' is greater than G''.

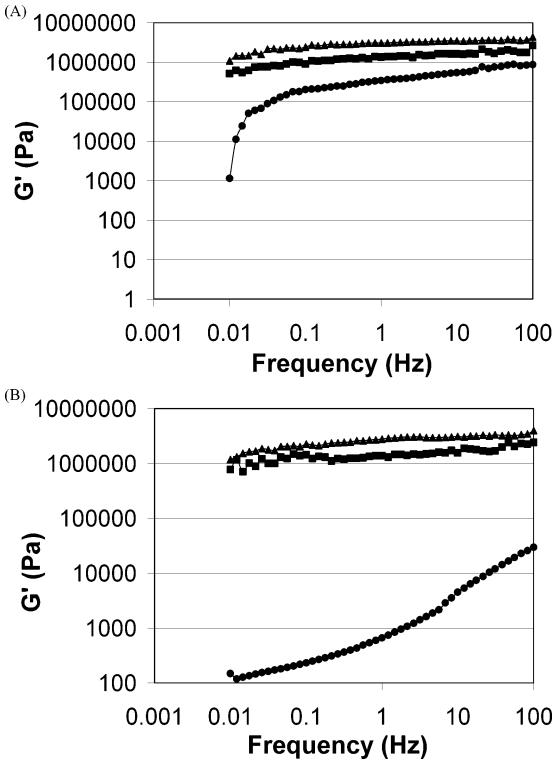


Figure 6. Frequency sweeps of purely physical gelation (●), purely chemical gelation (■), and physical−chemical gelation (▲) for copolymer 1 (A) and copolymer 2 (B). Modulus of physical gel in part A is elevated due to the presence of disulfide bonding.

with PEGDA (MW 575), mixed for 10 s, and placed on the rheometer at 20 °C ($n = 3$). The copolymers were considered gels when G' became greater than G''. The gel time of copolymer 1 is 8 ± 3 min, while the gel time of copolymer 2 is 10 ± 5 min (these gel times take into account the time required for mixing and placing the material on the rheometer) ($p > 0.05$). The amount of time required for copolymer 2 to form a gel was not shown to be significantly different than that of copolymer 1. With less thiols present, a greater percent of the thiols must form cross-links in order to create a gel, which should result in longer gel times. However, because the pK_a of copolymer 1 is greater than that of copolymer 2, a smaller percentage of thiols become thiolates at low pH; thus, a smaller percentage of thiolates are available for reaction and gel times are increased. Before gelation, both copolymers were flowable and able to be easily pushed through a syringe. After gelation, both copolymers formed strong gels, as apparent by storage moduli greater than 1 MPa.

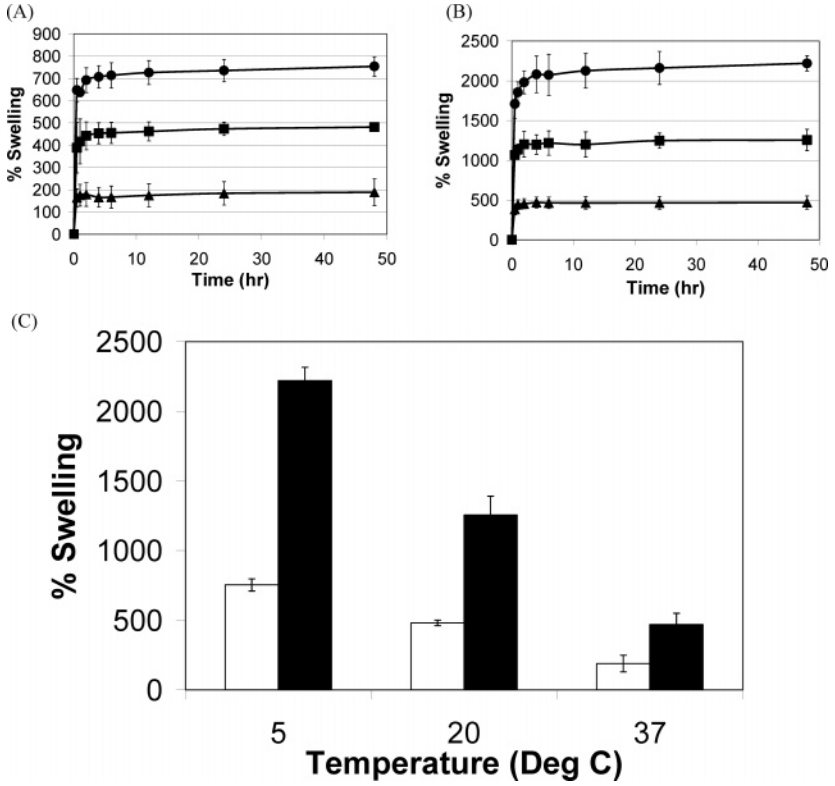


Figure 7. (A) Percent swelling vs time for copolymer 1 at various temperatures (5 °C, ●; 20 °C, ■; 37 °C, ▲) (B) Percent swelling vs time for copolymer 2 at various temperatures (5 °C, ●; 20 °C, ■; 37 °C, ▲). (C) Equilibrium percent swelling for both copolymers at various temperatures (copolymer 1, white bars; copolymer 2, black bars). ($n = 3$ for all).

Figure 6 shows the frequency sweeps on the physical gel, the chemical gel, and the physical−chemical gel for both copolymers. Figure 6A shows these sweeps for copolymer 1 and Figure 6B shows these sweeps for copolymer 2. As described previously, physical gels, formed by secondary forces including chain entanglement, hydrophobic interaction, ionic interaction, and Van der waals forces, are easily injected. However, such sol−gel systems based on purely physical cross-linking exhibit viscoelastic flow under constant, low-frequency stress.[7,11] This low-frequency strength loss due to viscoelasticity can be seen for both copolymers. Although the dynamic modulus of copolymer 1 increases to much larger values beyond frequencies of 0.10 compared to copolymer 2, this is believed to be due to disulfide bonding that occurred during dissolution, thus creating a partially chemical gel. The same response is not observed for copolymer 2, because of the decreased probability of disulfide bonding due to the reduced number of thiols present. Unlike physical gels, chemically cross-linked gels formed by covalent bonds result in stronger, less viscoelastic solids.[22] When the frequency response of the purely chemical gel is compared to that of the physical−chemical gel, it is apparent that the physical−chemical gel is stronger than that of the purely chemical gel. This is true for both copolymers. This increase in strength occurs because both the physical and chemical components of the polymer system are contributing to gel formation. The maximum values of G' for both copolymers are very similar. This is attributed to decreased cross-linking efficiency in copolymer 1. As described previously, injection of hydrophobic chemically cross-linked gels can be difficult due to gelation in the catheter, while hydrophilic chemically cross-linked gels can have high swelling and low mechanical properties.[11] The simultaneous system presented here remains flowable prior to injection and then immediately physically gels upon injection due to a temperature increase. If properly designed, this physical gel can be delivered through a catheter but washout prevented in the vessel temporarily. The system then cures quickly through chemical gelation to create a strong material with increased low-frequency load bearing.

Figure 7 shows the swelling test of both copolymers, chemically cross-linked with PEGDA at a thiol to acrylate ratio of 1:1. Figure 7A shows the swelling of copolymer 1 at temperatures of 5, 20, and 37 °C. As the temperature of the solution is increased, the extent of swelling of the polymer gel decreased due to the physical association of NIPAAm. At 5 °C, the gel swelled 750%, while at 20 and 37 °C, the polymer gel swelled about 480 and 188%, respectively ($p < 0.05$). This result is similar to that of other temperature-sensitive gels.[11] Figure 7B shows the swelling of copolymer 2 at temperatures of 5, 20, and 37 °C ($p < 0.05$). The same trend of increased swelling with decreased temperature is observed. In Figure 7C, both copolymers are compared to one another at each temperature. The percent swelling of copolymer 2 is greater than that of copolymer 1 at all temperatures, because of the decreased cross-linking present in copolymer 2 ($p < 0.05$). With fewer thiols present for reaction with acrylate, the cross-link density is decreased, resulting in increased swelling.

Conclusion

A simultaneously physically and chemically cross-linking polymer system was developed by synthesizing a poly(NIPAAm-*co*-cysteamine) copolymer and mixing it with a difunctional acrylate compound (PEGDA) in aqueous solutions. This tandem system displays two distinct gelation behaviors, where physical gelation is triggered by temperature increases above the LCST and chemical gelation occurs simultaneously through Michael-type addition reactions between the thiol groups and the

bifunctional acrylate groups in the PEGDA. While purely physical gels show poor low-frequency load bearing properties at physiological temperatures, additional chemical gelation greatly improved these properties. Additionally, the kinetics of the Michael-type addition reaction are highly dependent on pH, the ratio of thiol to acrylate, and the pK_a of the polymer. For this particular system, swelling is highly temperature-dependent and is directly related to the amount of cross-linking in the gel. Each of these properties may make this tandem polymer system a useful biomaterial for in situ-gelling applications, including functional embolization for arteriovenous malformations and aneurysms.

Acknowledgment. The authors acknowledge funding from the National Institutes of Health, Grant No. GM065917, and the Reach for the Stars Fellowship from the Arizona State University. The author would like to thank Dr. Michael Caplan and Dr. Stephen Massia for their helpful discussions and mentoring.

Supporting Information Available. Kinetic studies of disulfide bond formation for copolymer 2 at various pH values and of the Michael-type addition reaction between thiols and acrylates for copolymer 2 at various pH values. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Vernon, B. L.; Tirelli, N.; Bachi, T.; Haldimann, D.; Hubbell, J. A. Water-borne, in situ crosslinked biomaterials from phase-segregated precursors. *J. Biomed. Mater. Res.* **2002**, *64*, 447−456.
(2) McLemore, R.; Preul, M. C.; Vernon, B. L. Controlling delivery properties of a waterborne, in-situ-forming material. *J. Biomed. Mater. Res. B* **2006**, *79*, 398−410.
(3) Shu, X. Z.; Liu, Y.; Palumbo, F. S.; Luo, Y.; Prestwich, G. D. In situ crosslinkable hyaluronan hydrogels for tissue engineering. *Biomaterials* **2003**, *25*, 1339−1348.
(4) Zhao, C.; Wang, Q.; Meng, J.; Liu, W.; Liu, Z. A new thermosensitive polymer as noadhesive liquid embolism material. *Curr. Appl. Phys.* **2005**, *5*, 497−500.
(5) Becker, T.; Kipke, D.; Preul, M.; Bichard, W.; McDougall, C. In vivo assessment of calcium alginate gel for endovascular embolization of a cerebral arteriovenous malformation model using the swine rete mirabile. *Neurosurgery* **2002**, *51*, 453−458.
(6) Li, X.; Liu, W.; Ye, G.; Zhang, B.; Zhu, D.; Yao, K.; Liu, Z.; Sheng, X. Thermosensitive *N*-isopropylacrylamide-*N*-polyacrylamide-vinyl pyrrolidone terpolymers: Synthesis, characterization, and preliminary application as embolic agents. *Biomaterials* **2005**, *26*, 7002−7011.
(7) Ruel-Gariepy, E.; Leroux, J. C. In situ-forming hydrogels-review of temperature-sensitive systems. *Eur. J. Pharm. Biopharm.* **2004**, *58* (2), 409−426.
(8) Gutowska, A.; Jeong, B.; Jasionowski, M. Injectable gels for tissue engineering. *Anatom. Record* **2001**, *263*, (4), 342−349.
(9) Jeong, B.; Kim, S. W.; Bae, Y. H. Thermosensitive sol-gel reversible hydrogels. *Adv. Drug Delivery Rev.* **2002**, *54* (1), 37−51.
(10) Jeong, B.; Gutowska, A. Lessons from nature: stimuli responsive polymers and their biomedical applications. *Trends Biotechonol.* **2002**, *20*, (7), 305−311.
(11) Lee, B. H.; West, B.; McLemore, R.; Pauken, C.; Vernon, B. L. An in situ injectable physically and chemically gelling NIPAAm-based copolymer system for emobilization. *Biomacromolecules* **2006**, *7*, 2059−2064.
(12) Heggli, M.; Tirelli, N.; Zisch, A.; Hubbell, J. A. Michael-type addition as a tool for surface functionalization. *Bioconjugate Chem.* **2003**, *14*, 967−973.
(13) Lutolf, M. P.; Hubbell, J. A. Synthesis and physiochemical characterization of end-linked poly(ethylene glycol)-*co*-peptide hydrogels formed by Michael-type addition. *Biomacromolecules* **2003**, *4*, 713−722.
(14) Cellesi, F.; Tirelli, N.; Hubbell, J. A. Towards a fully-synthetic substitute of alginate: Development of a new process using thermal gelation and chemical crosslinking. *Biomaterials* **2003**, *25*, 5115−5124.
(15) Yang, H. J.; Cole, C. A.; Monji, N.; Hoffman, A. S. Preparation of a thermally phase-separating copolymer, poly(*N*-isopropylacrylamide-*co*-*N*-acryloxysuccinimide), with a controlled number of active esters per polymer chain. *J. Polym. Sci. Part A: Polym. Chem.* **1990**, *28* (1), 219−226.
(16) Ellman, G. L. A Colorimetric method for determining low concentrations of mercaptans. *Arch. Biochem. Biophys.* **1958**, *74*, 443−450.
(17) Lutolf, M. P.; Tirelli, N.; Cerritelli, S.; Cavalli, L.; Hubbell, J. A. Systematic modulation of Michael-type reactivity of thiols through the use of charged amino acids. *Bioconjugate Chem.* **2001**, *12*, 1051−1056.
(18) Shu, X. Z.; Ghosh, K.; Liu, Y.; Palumbo, F. S.; Luo, Y.; Clark, R. A.; Prestwich, G. D. Attachment and spreading of fibroblasts on an RGD peptide-modified injectable hyaluronan hydrogel. *J. Biomed. Mater. Res. A* **2004**, *68*, 365−375.
(19) Alarcon, C. H.; Pennadam, S.; Alexander, C. Stimuli responsive polymers for biomedical applications. *Chem. Soc. Rev.* **2005**, *34*, 267−285.
(20) Weng, Y.; Ding, Y.; Zhang, G. Microcalorimetric investigation on the lower critical solution temperature behavior of *N*-isopropylacrylamide-*co*-acrylic acid copolymer in aqueous solution. *J. Phys. Chem. B* **2006**, *110*, 11813−11817.
(21) Danehy, J. P.; Parameswaran, K. N. Acidic dissociation constants of thiols. *J. Chem. Eng. Data* **1968**, *13*, 386−389.
(22) Hoffman, A. S. Hydrogels for biomedical applications. *Adv. Drug Delivery Rev.* **2002**, *43*, 3−12.

BM070267R