Synthesis and Characterization of an Injectable Hydrogel with Tunable Mechanical Properties for Soft Tissue Repair

Emily Ho, Anthony Lowman, and Michele Marcolongo*

Department of Materials Science and Engineering, Drexel University, 3141 Chestnut Street, Philadelphia, Pennsylvania 19104

Received March 17, 2006; Revised Manuscript Received June 19, 2006

Injectable polymers are attractive materials for the fixation or augmentation of soft tissues. Thermosensitive hydrogels, especially poly(*N*-isopropylacryamide), have been investigated for these applications to exploit the lower critical solution temperature (LCST) which falls between room and body temperatures. One limitation to the material is the ability to withstand loading. In this work, we evaluated an injectable material system, poly-(*N*-isopropylacryamide)-*co*-poly(ethyleneglycol) dimethacrylate, with the addition of trimethacryloxypropyltrimethoxysilane (MPS). Our goal was to investigate the potential to tune the mechanical behavior of the injectable hydrogel. Addition of MPS to the hydrogel increased the compressive modulus but did not affect the LCST of the hydrogel. An increase in ion concentration of the immersion media resulted in less solution uptake by the hydrogels, regardless of MPS presence in the system. The challenge of this material system is to balance the network-forming and modulus-enhancing MPS while maintaining an injectable hydrogel for potential soft tissue repair.

1. Introduction

Many hydrogels have recently been developed as biomaterials for applications in the medical and pharmaceutical fields. Research studies have showed that the swelling behavior of hydrogels depends on the external environment.[1,2] They can exhibit abrupt changes in the swelling behavior of the network structure, permeability, or mechanical strength in response to changes in pH, ionic strength, temperature, and electromagnetic radiation. The most commonly studied hydrogels having environmental sensitivity are responsive to either pH or temperature.[3,4] Poly(*N*-isopropylacryamide) (PNIPAAm) is a thermosensitive hydrogel that has received much attention for biomedical use because of its lower critical solution temperature (LCST) behavior at around 32 °C in an aqueous solution.[5−8] PNIPAAm chains hydrate to form expanded structures in water when the solution temperature is below its LCST but become a compact gel structure by dehydration when heated to a temperature above its LCST. Below its LCST, PNIPAAm is extremely soluble in water and appears transparent. However, as its temperature is increased above the LCST, it becomes hydrophobic from the increased interactions between the isopropyl groups and PNIPAAm precipitates out from the aqueous solution, appearing opaque. PNIPAAm hydrogels possess a three-dimensional network structure which is insoluble but has characteristics of reversible swelling.[2] The polymer chains undergo a coil (soluble)−globule (insoluble) transition when the external temperature cycles across its LCST at about 33 °C.[1,9] Thus, at a temperature below the LCST, PNIPAAm hydrogels absorb water and exist in a swollen state but shrink and display an abrupt volume decrease when the environmental temperature is higher than the LCST. With this quick response to phase transition at body temperature, PNIPAAM may be an excellent candidate for injectable soft tissue replacement.

* To whom correspondence should be addressed. Phone: +1+215-895-2329. E-mail: marcolms@drexel.edu.

While the injection potential of PNIPAAm is attractive for use as a biomaterial, there are limitations to the material such as high deswelling upon phase transition and low compressive modulus. To overcome these properties, researchers have modified the polymer. At temperatures above the LCST of PNIPAAm (about 32 °C), the hydrogel expels water and can lose its gel behavior (such as elasticity). Kono et al.[10] demonstrated that with addition of poly-ethyleneglycol (PEG), a hydrophilic component, there was enhancement of elasticity and swellability of the gel network in body fluids. However, PEG is known for its inert behavior toward biosystems in general and protein adsorption in particular. Optimization of the hydrophobic and hydrophilic components is the key factor of this gel system. Above the phase-transition temperature, the hydrophobic surface increases, allowing for cell attachment, spreading, and proliferation, while the hydrophilic component (mostly from PEG) may initiate cell detachment.[11−13] PEG can be modified with dimethacrylates (PEGDM), and these materials have been shown to be biocompatible with the unreacted dimethacrylates, having a relatively low cytotoxicity.[14] Theoretically, each PEGDM chain has two functional side groups that would link with the two PNIPAAM chain and form a cross-linked copolymer system (Figure 1). With the additional degree of cross-linking, the mechanical properties of the polymer would be enhanced. Malonne et al. evaluated the in vivo toxicity of PNIPAAm and two copolymers: PNIPAAm-*co*-*N*-vinylacetamide (*co*-*N*-vinylacetamide = -NVA) and PNIPAAm-*co*-AAc.[15] Their acute and subacute study on mice indicated the absence of cumulative toxicity and a no-observed-adverse-effect level of PNIPAAm, PNIPAAm-*co*-NVA, and PNIPAAm-*co*-AAc.

Stile et al.[16] created loosely cross-linked polymers of PNIPAAm and PNIPAAm-*co*-acrylic acid as an injectable hydrogel for cartilage tissue applications. With addition of acrylic acid (AAc), the LCST of polymer increased about 2−34 °C. The PNIPAAm-*co*-AAc did not experience the same dramatic water loss as the PNIPAAm control. In fact, the initial water content at physiological temperature actually increased slightly but decreased

10.1021/bm0602536 CCC: $33.50
Published on Web 09/27/2006

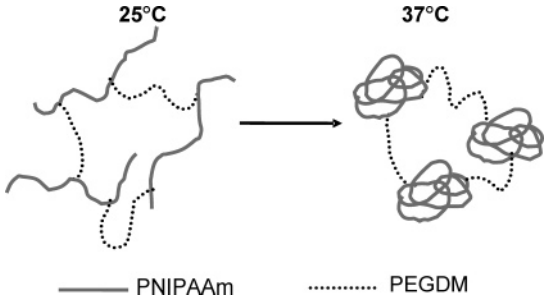

Figure 1. Simplified illustration of the polymer molecular structure of PNIPAAm-PEGDM at 25 and 37 °C.

to about 74% after 6 days in phosphate-buffered saline. These values become important in the context of in situ gelation since a polymeric plug needs to adequately fill the cartilage defect void. If too much water separates from the hydrogel, the polymer might not yield a tight or secure fit and, consequently, would decrease the potential for tissue regeneration. In vitro experiments with bovine chondrocytes demonstrated that both polymers supported cell viability and allowed cells to produce extracellular matrix molecules. These initial results showed that hydrogels derived from PNIPAAm can provide a biocompatible matrix for applications such as cartilage repair. They also reported that the complex shear moduli of the PNIPAAm-*co*-(4 wt %) acrylic acid (AAc) hydrogels after immersion in PBS and ultrapurified water at 37 °C ranged from 0.07 to 0.10 kPa.

In addition, Kim and Stile demonstrated that the complex shear modulus of the PNIPAAm-*co*-AAc gels can be further improved (up to 0.12 kPa) by cross-linking with peptides.[17] Zhang et al. investigated the physical and mechanical properties of several UV photo-cross-linked PNIPAAm-*co*-*N*,*N*-dimethylacrylamide hydrogels. The authors measured the compressive modulus of this family of hydrogels in 22 °C distilled water and found it ranged from 5 to 8 kPa.[18] Recently, Ohya et al. studied PNIPAAm-gelatin system for potential tissue regeneration. The elastic modulus of this system was determined using atomic force microscopy. The elastic modulus of the 18 wt % PNIPAAm−72 wt % gelatin system was reported to be 244 ± 89 kPa after immersion in a cell culture medium,[19] which is by far the highest elastic modulus reported in the literature for a PNIPAAm-based hydrogel. The disadvantage of this PNIPAAm-containing material was an increase in viscosity such that it was no longer injectable. In summary, the reported mechanical properties of the PNIPAAm-based gels depend on the cross-linker concentration, testing temperature, and testing media. A summary of the mechanical properties of different PNIPAAm-based hydrogels is found in Table 1.

Silane contains silanol side groups and has been applied as an adhesive agent for binding two materials and also as a cross-linking agent for various polymers.[20−23] Through incorporating a silane with PNIPAAm, we proposed that silanol groups may condense into the siloxane, serving as a cross-linker to the polymer. With proper molecular improvement of the PNIPAAm hydrogel, a multifunctional polymer can be produced for hard or soft tissue repair applications. In this study, we synthesized PNIPAAm-PEGDM system which we cross-linked with 3-(methacryloxy)propyltrimethoxysilane (MPS). We characterized the chemistry, water retention, swelling behavior, LCST, viscosity, and mechanical behavior of the injectable hydrogel system as a function of the MPS concentration. With proper molecular improvement of the PNIPAAm hydrogel, a multifunctional polymer can be produced for soft tissue repair.

2. Experimental Section

Materials. *N*-Isopropylacrylamide (NIPPAm) was obtained from Sigma (St. Louis, MO) and purified in hexane (Sigma-Aldrich Co., St. Louis, MO). Nitrogen gas was bubbled through a mixture of purified NIPAAm, PEGDM (Sigma-Aldrich Co.) in 200:1 weight ratios (about 0.05 mol % of PEGDM) in methanol (Sigma-Aldrich Co.). Various molar ratios of MPS:NIPAAm (0−0.01) and a constant molar ratio of calcium chloride:MPS of 2:1 was added to the polymer mixture. For each mole of NIPPAm, 0.05 mol of 2′-azobis-isobutyronitrile (Sigma-Aldrich Co.) was added to initiate polymerization. The mixture was polymerized at 65 °C for either 6 or 48 h and stirred overnight at room temperature. The polymer was dried for a few days in a vacuum oven to further remove the solvent, and then the dried PNIPAAm-MPS-PEGDM was ground to a fine powder using a grounder (Bel-Art products; Pequanock, NJ) for 3 min. Polymer solution was prepared with 25 wt % polymer and 75 wt % deionized (DI) water at room temperature.

The polymer solution was poured into a 10 mm diameter polymer cylinder, and the mold was heated to 37 °C solution for phase transformation. Then the firm gels were stored in the 37 °C immersion media (phosphate-buffered saline, PBS, and simulated body fluid, SBF) for 5 days to reach its equilibrium state. In the preparation of PBS, 9.6 g of Dulbecco's PBS powder (Sigma-Aldrich Co.) was dissolved in DI water. The simulated body fluid (SBF) contains ions to represent the ionic concentrations of plasma: 2.6 mM Ca^{2+} as $CaCl_2$, 1 mM HPO_4^{2-} as $K_2HPO_4{\cdot}3H_2O$, 152 mM Na^+ as NaCl, 135 mM Cl^{2+} as $CaCl_2$, 5 mM K^+ as KCl, 1.5 mM Mg^{2+} as $MgCl_2{\cdot}6H_2O$, 27 mM HCO^{3-} as $NaHCO_3$, and 0.5 mM SO_4^{2-} as $MgSO_4{\cdot}7H_2O$.[24,25] The chemicals were dissolved in deionized water and buffered to pH 7.6 at 37 °C with 1.0 mN Tris-HCl (Sigma-Aldrich Co.).[25]

Polymer Characterization. After synthesis, the polymers with various MPS contents were characterized using Fourier transform infrared spectroscopy (FTIR; Magna-IR 560, Nicolet, Madison, WI) The polymer powder was dissolved in acetone (Sigma-Aldrich Co.), which was poured onto the liquid crystal cell and allowed the acetone to be evaporated. The remaining polymer film was analyzed.

LCST Determination. The LCST of the polymer solution (25 wt % of polymer in DI water) was evaluated using differential scanning calorimetry (DSC; DSC 2010, TA Instruments, New Castle, DE) as a function of MPS content. The samples were heated at 1 °C/min to 50 °C under nitrogen gas purge. Once a plot of heat flow vs temperature was obtained, the LCST was determined to be the temperature at the minimum heat flow point of the curve.

Initial Phase Transformation. The phase transformation behavior of PNIPAAm-PEGDM hydrogels with and without 0.05 mol % MPS during the initial 60 min was analyzed. The polymer solution was injected in a 30 °C preheated 10 mm diameter glass container and placed in a 37 °C water bath for complete phase transformation. The accumulative volume of water expelled from the polymer solution after phase transformation was measured. The expelled water was removed using a pipet and measured after 1, 5, 15, 30, and 60 min of phase transformation.

Injectability and Viscosity Studies. An injection process can be considered in two physical processes: (1) delivery of the injected materials in the implantation site and (2) infiltration of the injected materials with the existing biological environment.[26] The delivery of the bioactive hydrogel was examined qualitatively via injection of polymer solutions with various MPS molar ratios through a 20 gauge needle in 37 °C SBF manually with a moderate force. The in situ infiltration properties of the bioactive hydrogel were studied via the viscosity measurement of the polymer solution as a function of MPS molar ratio. The viscosity measurement of three polymer solutions (25 wt % of PNIAAm-PEGDM with either 0, 0.005, or 0.01 mol ratio MPS in deionized water) was performed using a dynamic stress rheometer (DSR; DSR-200 Rheometics, TA Instruments, New Castle, DE) with the cone/plate test setup at room temperature. The applied shear rate was 1 to 100 s^{-1} for all specimens.

Table 1. Summary of Mechanical Properties of Different PNIPPAm-Based Hydrogels

author	material	mechanical properties	testing conditions	reported values (kPa)
Stile and Healy[16]	PNIPAAm-*co*-(4 mol %) acrylic acid	complex shear modulus at 10 Hz	PBS and ultrapurified water at 37 °C	0.07−0.10
Stile and Healy[16]	PNIPAAm-*co*-(4 mol %) acrylic acid	complex shear modulus at 10 Hz	PBS and ultrapurified water at 22 °C	0.02−0.04
Zhang et al.[18]	PNIPAAm-*co*-*N*,*N*-dimethylacrylamide	compressive modulus	distilled water at 22 °C	5−28
Kim and Healy[17]	peptide-PNIPAAm-*co*-(4 wt %) acrylic acid	complex shear modulus at 10 Hz	ultrapurified water at 37 °C	0.10−0.12
Kim and Healy[17]	peptide-PNIPAAm-*co*-(4 wt %) acrylic acid	complex shear modulus at 10 Hz	ultra purified water at 22 °C	0.02−0.03
Ohya et al.[19, 30]	PNIPAAm-*co*-(20 wt/v %) gelatin	surface elastic modulus (AFM indentation)	distilled water at 37 °C	158−286

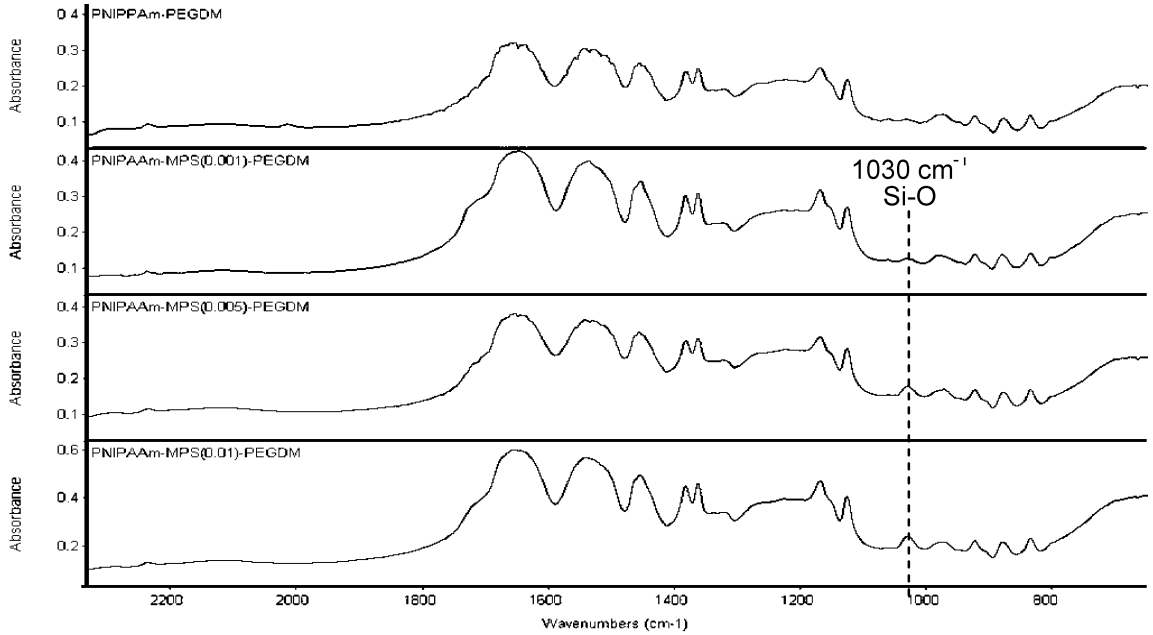

Figure 2. FTIR spectra of PNIPAAm-PEGDM with 0, 0.001, 0.005, and 0.01 mol of MPS.

In Vitro Compression Test. The hydrogel specimens were molded at 37 °C in either SBF or PBS. The total ion concentration of SBF is much higher than PBS. After the gel stabilized with the immersion solution, the effect of volume change of the gels on its mechanical properties would be negligible. The specimens were tested in unconfined compression using a mechanical testing system (Instron model 4442, Instron Co., Park Ridge, IL) fitted with a 50N load cell and 37 °C immersion bath with either PBS or SBF. Specimens were compressed at a strain rate of 100% strain per minute. Load and displacement data were recorded at 20 points per second with the Instron Series IX software. These data were converted to stress and strain values in Microsoft Excel using the specimen's initial dimensions. A compressive elastic modulus was measured as the initial linear slope of the stress−strain response at 0.05 mm/mm strain. Three SBF or PBS immersed specimens of PNIPAAm-PEGDM with either 0, 0.001, 0.003, 0.005, 0.0075, or 0.01 MPS molar ratio were mechanically tested. One-way ANOVA analyses were performed for compressive modulus results at 0.05 level of significance.

3. Results and Discussion

Synthesis of MPS−Cross-Linked PNIPAAm-PEGDM Hydrogel. Figure 2 shows the FTIR spectra of the gels with different chemistries. Comparing the FTIR spectra of MPS-free gels to MPS-containing gels, the peak at 1030 cm^{-1} (Si−O groups) appeared only in materials with a 0.005 and 0.01 mol ratio of MPS. The 0.001 mol ratio of MPS content in the gel was not detectable, likely due to the FTIR sensitivity limit (±1%).

Solubility Analyses. PNIPAAm-PEGDM polymer with 0.001, 0.003, 0.005, 0.0075, and 0.01 mol ratios of MPS was immiscible in water even after 14 days at room temperature. It is possible that the high degree of cross-linking from addition of MPS prevents the polymer from being soluble with water. Therefore, all reported material characterizations were performed on PNIPAAm-PEGDM with a 0−0.01 mol ratio of MPS content. Validation of different polymerization times (6 and 48 h) was performed by comparing the physical properties of the MPS-containing PNIPAAm-PEGDM polymer solutions, which included 25 wt % polymer powder in deionized water. The MPS-added polymer was expected to be lightly cross-linked and resulted in a highly viscous polymer solution. However, less viscous polymer solutions were obtained from 6 h polymerized MPS-added gels, and more viscous polymer solutions resulted from 48 h of polymerization. The dissimilarity in performance of the polymer solutions is related to the degree of cross-linking of the polymer. With less polymerization time (6 h), an incomplete cross-linked gel was most likely obtained.

On the basis of Table 2, MPS-free and 0.001 mol ratio MPS-containing polymer solutions became transparent within 2 days while the 0.003 and 0.005 mol ratio of MPS-containing polymer solutions became transparent after 7 and 14 days, respectively.

Table 2. Transparency Results for PNIPAAm−PEGDM-Based Hydrogels

mol ratio of MPS	DI water content (wt %)	$CaCl^-$ containing?	appearance at room temperature after 2 days	after 7 days	after 14 days
0	75	no	transparent	transparent	transparent
0.001	75	yes	transparent	transparent	transparent
0.003	75	yes	opaque	transparent	transparent
0.005	75	yes	opaque	opaque	transparent
0.005	75	no	transparent	transparent	transparent
0.0075	75	yes	opaque	opaque	opaque
0.01	75	yes	opaque	opaque	opaque

Moreover, the 0.0075 and 0.01 mol ratio MPS-containing polymer solutions remained opaque throughout 14 days in deionized water, which suggested that some degree of phase separation occurred in those polymer solutions. Meanwhile, the MPS-free and 0.005 mol ratio MPS-containing PNIPAAm-PEGDM polymers with no calcium chloride were miscible with deionized water within 2 days and transparent. With addition of calcium chloride, the polymer solution was opaque after 2 days of dissolving and became transparent slowly within 14 days at room temperature. The solubility of the gel in water was influenced by addition of calcium salts. The calcium salts may be less miscible with water and may structure around the polymer, therefore, extending the required time to solubility. In addition, the solubility of hydrogels in deionized water can be influenced by many other factors. For instance, reduced temperature (as low as 4 °C) can enhance the amount of hydrogen-bonding interactions between water and hydrophilic amide groups, causing the polymer to be miscible in water at a faster rate.[9] Decreased polymer particle size to a submicrometer level can increase the overall surface area for polymer−water, which can improve the solubility of the polymer in water.

LCST and Viscosity. No significant change was measured on the LCST of the PNIPAAm-PEGDM gels regardless of MPS concentration ($p = 0.62$), as shown in Figure 3. Consequently, polymer solutions (with up to 0.01 mol ratio of MPS) transformed to firm gels at about 32 °C (the LCSTs). Ease of injection through a 20-gauge needle was accomplished with MPS concentrations of 0.01 mol ratio or less. The gel became solid instantaneously in 37 °C SBF (Figure 4). On the basis of the DSR data, the viscosity measurements (η) of the polymer solution of 0, 0.005, and 0.01 mol ratio of MPS with respect to a shear rate of 10−100 s^{-1} are plotted in Figure 5. The room-temperature-measured viscosity of each polymer solution reduced with increasing shear rate and reached an equilibrium level at 80 s^{-1} shear rate. The equilibrium viscosity of 25 wt % PNIPAAm-PEGDM and DI water solution with 0, 0.005, and 0.01 mol ratio of MPS content, at a shear rate of 100 s^{-1}, was determined to be 3, 118, and 83 Poise, respectively. Arai et al. reported that PNIPAAM solution presents such a strong thermoviscosifying effect that its viscosity increased from 0.5 to 100 Poise at its LCST 32 °C due to the rapid phase transformation of the gel.[27] The 0.01 mol ratio of MPS-containing polymer solution had a lower viscosity than 0.005 mol ratio of MPS.

When gels were heated to 37 °C, all hydrogels became opaque and pliable in SBF. However, once they were immersed in SBF for 24 h, all hydrogels turned transparent and more rigid. During the rapid phase transition at 37 °C the hydrogels dramatically collapsed and released a large fraction of pore water within the first hour of heating. Then the specimens tended to swell (increase in volume) and reach equilibrium (volume change within 10%).

Phase Transformation Behavior. The polymer solution completed its phase transformation to a firm gel within 3 min in a 37 °C water bath. The percentage of accumulative volume of the water expelled from phase-transformed gels as a function of immersion time is plotted in Figure 6. The accumulative volume of water became steady, and no significant additional volume of water expelled from the gels after 15 min of immersion. The MPS-containing gels showed a higher volume of expelled water than the MPS-free gel after 15 min of immersion. The presence of MPS in the PNIPAAm-PEGDM gel enhances cross-linking in the system, which may affect the phase transformation behavior and the amount of water expelled in the gel at 37 °C. Nevertheless, both specimens demonstrated a rapid polymer setting and reached a stable volume within a relatively short period of time after immersion (about 15 min). For the compositional ranges examined, no significant influence was observed with MPS and PEGDM content on the phase-transition behavior and LSCT of the hydrogels. However, the enthalpy of transition of 0.01 mol ratio of MPS-containing PNIPAAm-PEGDM gel (8.28 J/g) was about 23% higher than that of 0.005 mol ratio of MPS-containing gel (10.85 J/g) and MPS-free gel (10.72 J/g), see Figure 3. The hydrogel with higher integrated heat flow may contain a higher degree of cross-linking. Alvarez-Lorenzo et al. remarked that the increase in the post-cross-linking degree in the PNIPAAm-chitosan copolymer caused a decrease in the enthalpy of the transition.[28] With an increment in the degree of cross-linking, the swellability of the polymer would be reduced[29] due to reduction of the polymer network mesh size.

An instantaneous phase transition and rapid dehydration of the gels at 37 °C in SBF was demonstrated. This sharp transition from the balancing hydrophobic/hydrophilic groups of the PNIPAAm is consistent with previous reports of injecting PNIPAAm in water and PBS at 35 °C.[1,9] Such rapid solidification of the polymer solution in simulated biological conditions led to the motivation to use PNIPAAm-PEGDM with MPS as an injectable soft tissue material. Even though rapid dehydration of the gel would lead to quick deswelling of the material in SBF, the volume did not change more than 10% after the first hour of injection. This factor is beneficial for soft and hard tissue replacements because it offers a physically stabilized material in the body first hour of surgery that would reduce the overall surgical risk since it could be injected into a surgical site in a minimally invasive manner.

In Vitro Mechanical Behavior. Figure 7 illustrates the elastic moduli of PNIPAAm-PEGDM as a function of MPS molar ratio after immersion in either PBS or SBF for 5 days. With addition of 0.005 mol ratio of MPS, the elastic modulus of the PBS-immersed firm gel had a 203% increase over PNIPAAm alone and reached 0.18 MPa, while the SBF-immersed one had a 293% increase to about 0.6 MPa. However, the elastic modulus of SBF-immersed gel with 0.0075 and 0.01 mol ratio of MPS had a lower value than the one with 0.005 mol ratio of MPS (132% and 89% less, respectively). Thus, the maximum elastic modulus of the bioactive gels was achieved with addition of 0.005 mol ratio of MPS after 5 days of immersion in either SBF or PBS.

With addition of cross-linker (MPS) to 0.005 mol ratio, the gels were likely to have a higher degree of cross-linking and result in deswelling of the gels after immersion in various media. MPS contributed to increased viscosity and elastic modulus of the material, likely the result of condensation of silanol, which

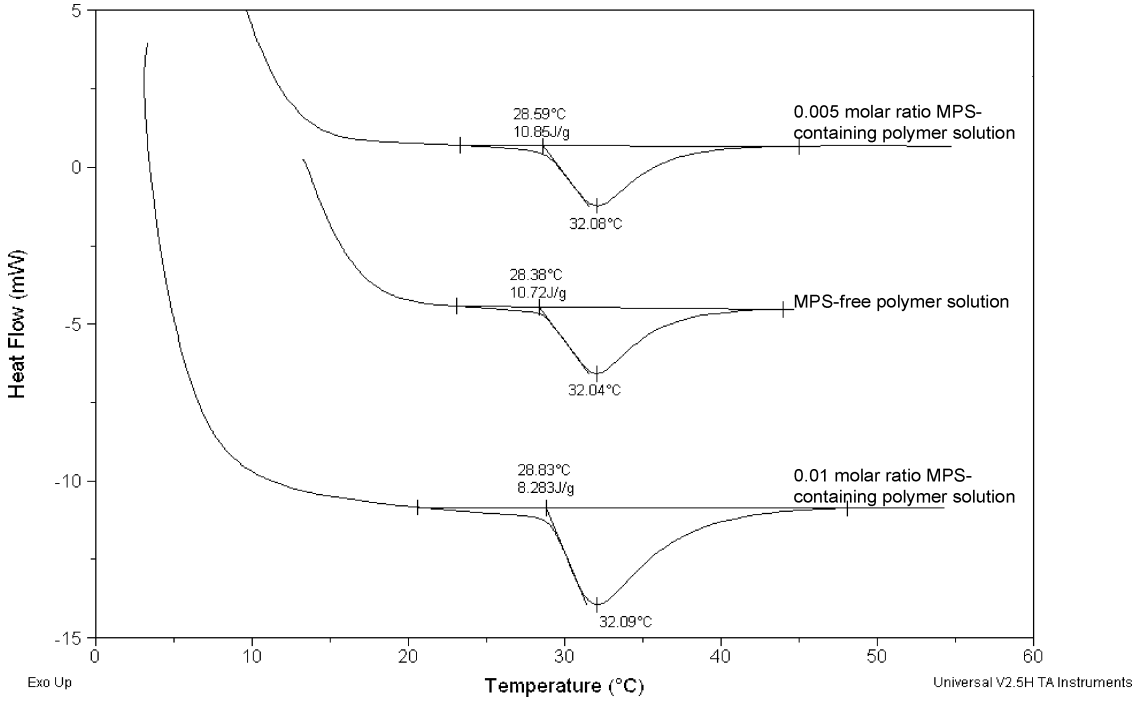

Figure 3. Observations of the physical properties of polymer solutions of either 6 or 48 h polymerized PNIPAAm−MPS-PEGDM with various MPS content (from 0 to 0.01 mol ratio).

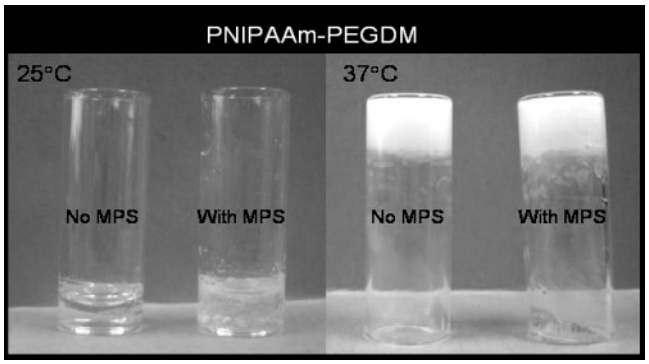

Figure 4. Phase transformation of PNIPAAm-PEGDM with and without MPS. The glass container was placed upside down to show that both polymers were solid and adhered to the glass container.

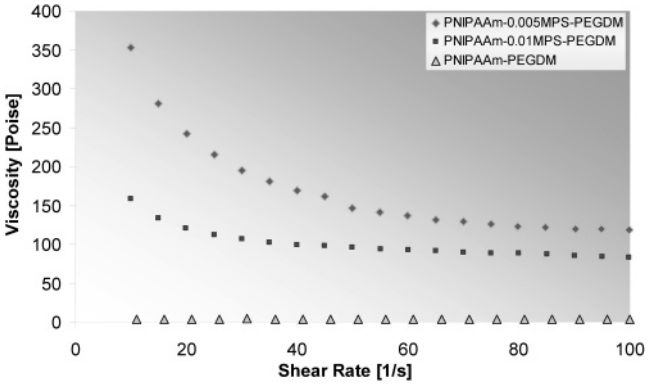

Figure 5. Viscosity measurements of PNIPAAm-PEGDM with 0, 0.005, and 0.01 MPS mol ratio with respect to shear rate from 10 to 100 s^{-1}.

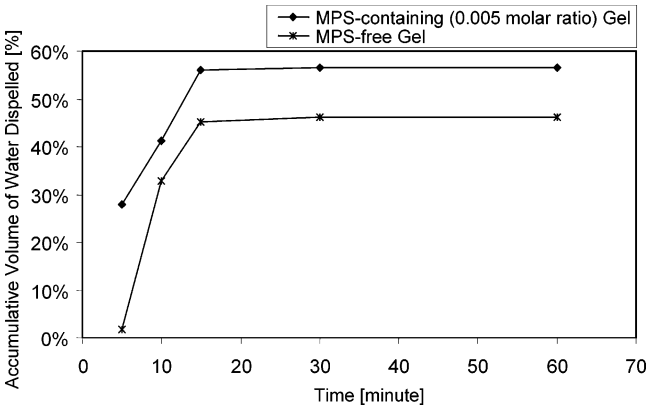

Figure 6. Percentage of accumulative water expelled volume during the initial 60 min of immersion in a 37 °C water bath.

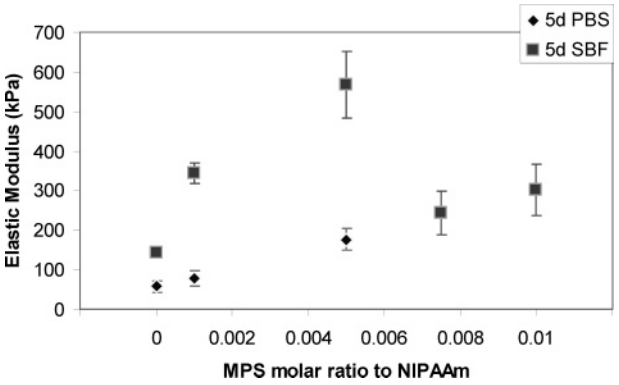

Figure 7. Graph of elastic compressive moduli of the PNIPAAm-PEGDM systems ($n = 3$) as a function of MPS molar ratio after immersion in PBS or SBF for 5 days.

forms siloxane (Si−O−Si), serving to cross-link the polymer backbone. In theory, the elastic modulus is expected to increase with the MPS content; however, the elastic moduli of the gels with 0.0075 and 0.01 mol ratio of MPS were lower than the one containing 0.0005 mol ratio of MPS. The gels with MPS more than 0.005 mol ratio may have poor distribution of siloxane bonds, and high content of siloxane bonds can lead to formation of a structure similar to brittle silica glass. Figure 7 shows that the differences in elastic moduli are more pronounced in the presence of SBF. As compared to PBS, immersion in SBF served to increase the mechanical properties, even after 5 days

of immersion in vitro. As previously mentioned, the SBF-immersed gels likely exhibited more cross-linking from the higher ions concentration of SBF than the PBS-immersed ones. With higher degree of cross-linking, superior elastic modulus resulted. Also, the SBF-immersed polymer has shown less swelling in 37 °C solution;[29] reduced solution content may result in an increment of the elastic modulus.

4. Conclusion

With addition PEGDM and MPS, the compressive modulus of the PNIPAAm systems reached 0.6 MPa, which is the highest modulus value currently reported for an injectable hydrogel biomaterial. The challenge of PNIPAAm-MPS-PEGDM systems is to balance the network-forming MPS with the gain in elastic recovery induced by PEGDM addition to PNIPPAAm, all while maintaining an injectable material system. We have shown that this hydrogel family is capable of "structural" mechanical behavior. Meanwhile, it is necessary to investigate the biocompatibility and potential bioactivity of the MPS-containing hydrogels. Future work would focus on selecting the appropriate permutations for this system that would lead to even higher modulus materials which closely match the modulus of either soft or hard biological tissues.

References and Notes

(1) Andersson, M.; Axelsson, A.; Zacchi, G., Swelling kinetics of poly-(N-isopropylacrylamide) gel. *J. Controlled Release* **1998**, *50* (1−3), 273−281.

(2) Kost, J.; Langer, R. Responsive polymeric delivery systems. *Adv. Drug Delivery Rev.* **1991**, *6* (1), 19−50.

(3) Hoffman, A. S. Hydrogels for biomedical applications. *Adv. Drug Delivery Rev.* **2002**, *54* (1), 3−12.

(4) Kopecek, J. Smart and genetically engineered biomaterials and drug delivery systems. *Eur. J. Pharm. Sci.* **2003**, *20* (1), 1−16.

(5) Huang, J.; Wang, X.-L.; Qi, W.-S.; Yu, X.-H. Temperature sensitivity and electrokinetic behavior of a N-isopropylacrylamide grafted microporous polyethylene membrane. *Desalination* **2002**, *146* (1−3), 345−351.

(6) Ju, H. K.; Kim, S. Y.; Lee, Y. M. pH/temperature-responsive behaviors of semi-IPN and comb-type graft hydrogels composed of alginate and poly(N-isopropylacrylamide). *Polymer* **2001**, *42* (16), 6851−6857.

(7) Kim, J. H.; Lee, S. B.; Kim, S. J.; Lee, Y. M. Rapid temperature/pH response of porous alginate-*g*-poly(N-isopropylacrylamide) hydrogels. *Polymer* **2002**, *43* (26), 7549−7558.

(8) Kim, S. J.; Park, S. J.; Kim, S. I. Synthesis and characteristics of interpenetrating polymer network hydrogels composed of poly(vinyl alcohol) and poly(N-isopropylacrylamide). *React. Funct. Polym* **2003**, *55* (1), 61−67.

(9) Tanaka, T. Kinetics of phase transition in polymer gels. *Physica A* **1986**, *140* (1−2), 261−268.

(10) Kono, K.; Yoshino, K.; Takagishi, T. Effect of poly(ethylene glycol) grafts on temperature-sensitivity of thermosensitive polymer-modified liposomes. *J. Controlled Release* **2002**, *80* (1−3), 321−332.

(11) Kwon, G. S.; Naito, M.; Kataoka, K.; Yokoyama, M.; Sakurai, Y.; Okano, T. Block copolymer micelles as vehicles for hydrophobic drugs. *Colloids Surfaces B: Biointerfaces* **1994**, *2* (4), 429−434.

(12) Neradovic, D.; Soga, O.; Van Nostrum, C. F.; Hennink, W. E. The effect of the processing and formulation parameters on the size of nanoparticles based on block copolymers of poly(ethylene glycol) and poly(N-isopropylacrylamide) with and without hydrolytically sensitive groups. *Biomaterials* **2004**, *25* (12), 2409−2418.

(13) Yasugi, K.; Nagasaki, Y.; Kato, M.; Kataoka, K. Preparation and characterization of polymer micelles from poly(ethylene glycol)-poly-(,-lactide) block copolymers as potential drug carrier. *J. Controlled Release* **1999**, *62* (1−2), 89−100.

(14) Lin-Gibson, S.; Bencherif, S.; Cooper, J. A.; Wetzel, S. J.; Antonucci, J. M.; Vogel, B. M.; Horkay, F.; Washburn, N. R. Synthesis and characterization of PEG dimethacrylates and their hydrogels. *Biomacromolecules* **2004**, *5* (4), 1280−1287.

(15) Malonne, H.; Eeckman, F.; Fontaine, D.; Otto, A.; Vos, L. D.; Moes, A.; Fontaine, J.; Amighi, K. Preparation of poly(N-isopropylacrylamide) copolymers and preliminary assessment of their acute and subacute toxicity in mice. *Eur. J. Pharm. Biopharm.* **2005**, *61* (3), 188−194.

(16) Stiles, R. A.; Healy, K. E. Poly(N-isopropylacrylamide)-based semi-interpenetrating polymer networks for tissue engineering applications effects of linear poly(acrylic acid) chains on phase behavior. *Biomacromolecules* **2002**, *3*, 591−600.

(17) Kim, S.; Healy, K. E. Synthesis and characterization of injectable poly(N-isopropylacrylamide-*co*-acrylic acid) hydrogels with proteolytically degradable cross-links. *Biomacromolecules* **2003**, *4* (5), 1214−1223.

(18) Zhang, X.; Wu, D.; Chu, C. C. Synthesis and characterization of partially biodegradable, temperature and pH sensitive Dex-MA/PNIPAAm hydrogels. *Biomaterials* **2004**, *25* (19), 4719−4730.

(19) Ohya, S.; Kidoaki, S.; Matsuda, T. Poly(N-isopropylacrylamide) (PNIPAM)-grafted gelatin hydrogel surfaces: interrelationship between microscopic structure and mechanical property of surface regions and cell adhesiveness. *Biomaterials* **2005**, *26* (16), 3105−3111.

(20) Wang, M.; Deb, S.; Bonfield, W. Chemically coupled hydroxyapatite-polyethylene composites: processing and characterisation. *Mater. Lett.* **2000**, *44* (2), 119−124.

(21) Wang, M.; Bonfield, W. Chemically coupled hydroxyapatite-polyethylene composites: structure and properties. *Biomaterials* **2001**, *22* (11), 1311−1320.

(22) Liu, L.; Sheardown, H. Glucose permeable poly (dimethyl siloxane) poly (N-isopropyl acrylamide) interpenetrating networks as ophthalmic biomaterials. *Biomaterials* **2005**, *26* (3), 233−244.

(23) Ohtsuki, C.; Miyazaki, T.; Tanihara, M. Development of bioactive organic−inorganic hydride for bone substitutes. *Mater. Sci. Eng.* **2002**, *22*, 27−34.

(24) Marcolongo, M.; Ducheyne, P.; Garino, J.; Schepers, E. Bioactive glass fiber/polymeric composites bond to bone tissue. *J. Biomed. Mater. Res.* **1998** (39), 161−170.

(25) Yao, J.; Radin, S.; S. Leboy, P.; Ducheyne, P. The effect of bioactive glass content on synthesis and bioactivity of composite poly (lactic-*co*-glycolic acid)/bioactive glass substrate for tissue engineering. *Biomaterials* **2005**, *26* (14), 1935−1943.

(26) Baroud, G.; Bohner, M.; Heini, P.; Steffon, T. Injection biomechanics of bone cements used in vertebroplasty. *Biomed. Mater. Eng.* **2004**, *00*, 1−18.

(27) Arai, F.; Ichikawa, A.; Fukuda, T.; Katsuragi, T. In *Continuous culture and monitoring of selected and isolated microorganisms on a chip by thermal gelation;* 7th International Conference on Miniaturized Chemical and Biochemical Analysis Systems, Squaw Valley, CA, 2003; Squaw Valley, CA, 2003.

(28) Alvarez-Lorenzo, C.; Concheiro, A.; Dubovik, A. S.; Grinberg, N. V.; Burova, T. V.; Grinberg, V. Y. Temperature-sensitive chitosan-poly(N-isopropylacrylamide) interpenetrated networks with enhanced loading capacity and controlled release properties. *J. Controlled Release* **2005**, *102* (3), 629−641.

(29) Ho, E.; Lowman, A.; Marcolongo, M. In situ apatite forming injectable hydrogel. *J. Biomed. Mater. Res.* **2006**, submitted for publication.

(30) Ohya, S.; Sonoda, H.; Nakayama, Y.; Matsuda, T., The potential of poly(N-isopropylacrylamide) (PNIPAM)-grafted hyaluronan and PNIPAM-grafted gelatin in the control of post-surgical tissue adhesions. *Biomaterials* **2005**, *26* (6), 655−659.

BM0602536