Published in final edited form as:

Macromol Biosci. 2014 May ; 14(5): 687–698.

Poly(ethylene glycol) Hydrogels with Adaptable Mechanical and Degradation Properties for Use in Biomedical Applications^a

Dr. Matthew Parlato,

Department of Biomedical Engineering, Wisconsin Institutes for Medical Research, University of Wisconsin Madison, 1111 Highland Ave., Madison, WI 53705, USA

Dr. Sarah Reichert,

Department of Biomedical Engineering, Wisconsin Institutes for Medical Research, University of Wisconsin Madison, 1111 Highland Ave., Madison, WI 53705, USA

Dr. Neal Barney, and

Department of Ophthalmology and Visual Sciences, Wisconsin Institutes for Medical Research, University of Wisconsin Madison, 1111 Highland Ave., Madison, WI 53705, USA

Dr. William L. Murphy

Department of Biomedical Engineering, Wisconsin Institutes for Medical Research, University of Wisconsin Madison, 1111 Highland Ave., Madison, WI 53705, USA, Department of Orthopedics and Rehabilitation, Wisconsin Institutes for Medical Research, University of Wisconsin Madison, 1111 Highland Ave., Madison, WI 53705, USA

William L. Murphy: wlmurphy@wisc.edu

Abstract

Requirements of hydrogels for drug delivery, wound dressings, and surgical implantation can be extensive, including suitable mechanical properties and tailorable degradation time frames. Herein, an adaptable PEG-based hydrogel, whose mechanical properties and degradation rate can be systematically adjusted to meet these criteria by altering simple variables such as the PEG molecular weight, is described. The performance of these hydrogels in three physical manipulations (pushing, pulling, and folding), representative of manipulations that they may undergo during typical biomedical use, is also assessed. While not all of these formulations can withstand these manipulations, a subset did, and it is intended to further optimize these formulations for specific clinical applications. Additionally, the outcomes of the physical manipulation tests indicate that simply having a high modulus does not correlate with biomedical applicability.

^aSupporting Information is available from the Wiley Online Library or from the author.

© 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Correspondence to: William L. Murphy, wlmurphy@wisc.edu.

Keywords

adaptable hydrogels; biomaterials; biomedical applications; hydrogels; poly(ethylene glycol)

1. Introduction

Hydrogels have been used in biomedical applications due to their hydrophilic and porous nature.^[1–10] In vivo applications have included use as wound dressings^[1,2,7] and tissue scaffolding.^[1,5] They have also been used as vehicles for drug delivery^[1,3,4] and cellular delivery.^[1,5,11,12] In these applications, hydrogel mechanical properties are consistently identified as an important property for successful use.^[1,2,7,13,14] However, hydrogels tend to have weak mechanical properties, such as a low modulus and low stress to failure.^[1–3,13] Hydrogels are often unable to withstand basic mechanical manipulations in clinical scenarios such as folding, pushing, and pulling during surgical implantation.^[1,13] Weak mechanical properties can also lead to formation of debris around the implant site upon hydrogel fracture or as a result of complete mechanical failure.^[13]

Degradable hydrogels are particularly susceptible to mechanical failure because most hydrogels degrade via bulk erosion and their modulus decreases during this degradation.^[15] Conversely, if a hydrogel degrades slowly, it can be a barrier to tissue in-growth thus hindering the healing process.^[16] Therefore, a need exists for hydrogels that degrade within predictable time frames so that degradation (and hence the loss of mechanical properties) can be tailored specifically to the time frame of needed mechanical support. More broadly, natural tissues have widely varying mechanical support needs and healing time frames, so a need exists for hydrogels whose properties can be tailored to meet this wide range of requirements.^[7,17]

Here, we present PEG-based hydrogels whose mechanical properties and degradation time frames can be systematically adjusted. We chose PEG as the base material for these hydrogels since hydrogels fabricated solely from PEG have shown some ability to have their bulk properties tailored through adjustments to the molecular weight and concentration of the PEG polymer.^[4,6,12,16,18–22] We have previously evaluated the degradation and erosion properties of these hydrogels, as well as their ability to function as an in vitro, 3D cell culture matrix.^[6] Given the adaptability of these hydrogels that our previous work demonstrated,^[6] we posit that these hydrogels may be able to be adapted to meet the vast array of requirements of hydrogels utilized in vivo. However, in our previous work we did not explicitly characterize many properties that are pertinent for in vivo utilization, such as their mechanical properties. Thus, we sought to more fully characterize these hydrogels with respect to both their mechanical and degradation properties.

These hydrogels are formed by first reacting a molar excess of poly(ethylene glycol)-diacrylate (PEGDA) with the dithiol molecule, dithiothreitol (DTT), in a step-growth polymerization reaction. After this reaction is complete, the remaining acrylate groups are photo-crosslinked to form a hydrogel network. While the poly(acrylate) photocrosslinks are stable and do not degrade within the course of this study, the esters proximal to the thioethers in the acrylate-thiol linkage are susceptible to hydrolysis, thus leading to a hydrolytically labile hydrogel network^[6] (Figure 1). Systematic adjustments in degradability and mechanical properties are brought about by modulating of the number of hydrolytically labile sites, PEG molecular weight, and PEG concentration (wt%).

While traditional characterization methods provide clear measurements of particular hydrogel parameters, it is difficult to predict hydrogel performance in more complex situations directly from these measures. For example, predicting whether a hydrogel could withstand being folded in half directly from idealized tension and compression measurements is difficult because of the complex interplay of these two parameters while the hydrogel is folding. Thus, aside from bulk characterization of these hydrogels, we also evaluated their performance in simple, mechanical manipulations that are representative of typical clinical manipulations. By correlating hydrogel performance in these physical manipulations to bulk hydrogel properties, we identify relationships between hydrogel parameters and potential clinical applicability. Our results identify a subset of hydrogel formulations that meet basic criteria for clinical applicability, and indicate that high modulus does not necessarily correlate with the ability to withstand physical manipulations.

2. Materials

2.1. PEGDA Synthesis

The required reagents for this synthesis are: anhydrous benzene, triethyl amine (TEA), acryloyl chloride, diethyl ether, and PEG of the desired molecular weight (M_n of 3.4, 8, and 12 kDa were used in this study). Anhydrous benzene, TEA, acryloyl chloride, and diethyl ether were obtained from Sigma–Aldrich. PEG of 3.4 and 8 kDa molecular weights were obtained from Sigma–Aldrich, and PEG of 12 kDa molecular weight was obtained from Alfa Aesar. PEG polymers were obtained in the alcohol-terminated form. Functionalization of the PEG polymer was verified via NMR. The main PEG peak appeared at 3.5 ppm, and the three acrylate peaks appeared between 5.5 and 6.5 ppm. Reaction efficiency was determined by comparing the signal/hydrogen ratio of the main PEG peak to the acrylate peaks.

2.2. PEG-Dithiol Reactions

The required reagents for this reaction are PEGDA and DTT. PEGDA of the desired molecular weight was synthesized as described above, and DTT was obtained from Sigma–Aldrich. Both reagents were dissolved in phosphate-buffered saline (PBS) that was obtained in powdered form from Fisher Scientific. PBS was described by Fisher Scientific as containing 81% sodium chloride, 14% sodium phosphate dibasic, 3% potassium phosphate monobasic, and 2% potassium chloride. The pH of the PBS was 7.4 for all chemical reactions and all hydrogel studies.

2.3. Hydrogel Formation

The required reagents for hydrogel formation are PBS, photo-initiator solution, and PEGDA or the PEG-DTT polymer. PBS was obtained in powdered form from Fisher Scientific (described in detail above). Photo-initiator solution was Irgacure 2959 (obtained from Ciba) dissolved in PBS at a concentration of 0.5% w/v.

3. Methods

3.1. PEGDA Synthesis

The procedure is similar to those that are well described elsewhere.^[4] Briefly, PEG (in the alcohol terminated form) was reacted with a fourfold molar excess of TEA and acryloyl chloride to form acrylate terminated PEG. The PEG was then precipitated in ethyl ether and recovered via filtration. PEGDA was purified via dialysis in cellulose ester membranes and functionalization was verified by NMR (as described in Section 2.1).

3.2. PEG-Dithiol Reactions

PEGDA of the desired molecular weight was dissolved in 7.4 pH PBS at the desired concentration (calculated as weight of polymer/volume of PBS). The desired amount of DTT was then dissolved, and the solutions were incubated at 37 °C for approximately 1 h. A more thorough description of this process is included in work by Hudalla et al.,^[6] and we include a more thorough description of this process in the Supporting Information, Methods.

PEG-dithiol reactions were characterized via five methods: the fluorimetric Measure-iT thiol assay available from Invitrogen Corporation to measure thiol presence at the reaction's end-point, UV-Vis spectroscopy measuring absorbance at 233 nm before and after the reaction, UV-Vis spectroscopy to measure the appearance or absence of disulfide bond absorbance at 283 nm, gel permeation chromatography (GPC) of the resulting PEG-DTT polymer (described in the Supporting Information, Methods), and NMR of the resulting PEG-DTT polymer. The Measure-iT thiol assay kit was obtained from Invitrogen Corporation, and micromolar reaction solutions (68.75 μ M solutions of PEG with the appropriate amount of dithiol) were analyzed. This kit operates on the basis of the assay compound binding to free thiol groups and then fluorescing (measured by 480 nm excitation and 520 nm emission with 20 and 15 nm band width filters, respectively). Measurements were made on reaction solutions after incubation, and the assay standard was glutathione. Remaining thiol was compared to the total original amount of thiol, and reaction efficiency was determined from this. *N* was equal to three for all conditions.

UV-Vis spectroscopy was performed at 233 nm to observe the disappearance of thiol and acrylate groups. Measurements of absorbance were taken before and after the reaction of the PEGDA and DTT species. PEG solutions were made at a concentration of 1.5 mM (in 7.4 pH PBS) with appropriate amounts of DTT dissolved in the reaction solutions. Absorbance measurements taken before and after incubation were back-calculated to acrylate and thiol concentrations and then used to calculate reaction efficiency. UV-Vis spectroscopy was also performed at 283 nm to observe disulfide bond formation. *N* was equal to three for all conditions.

NMR was performed to determine the molecular weight of the resulting PEG-DTT polymers. These polymers were dissolved in deuterated chloroform (containing 0.03% v/v tetramethyl silane as an internal standard) at a concentration of 10 mg mL⁻¹. Acrylate peaks emerged between 5.5 and 6.5 ppm, and the peaks of the repeating polymer units emerged around 3.5 ppm. Given that all polymers were ultimately terminated with acrylate groups and the number of hydrogen atoms per acrylate group is constant (three hydrogen atoms per group and two groups per polymer chain), the signal-per-proton value was obtained from these peaks. This value was used to convert the integral of the repeating unit peaks into the number of repeat units comprising the polymer. The repeating unit of a PEG polymer has a molecular weight of 44 g mol⁻¹, so this value was used to convert the number of repeat units into the molecular weight of the polymer.

3.3. Hydrogel Formation

All hydrogels were cast into Teflon molds and cured with UV light for approximately 7 min (365 nm at 4.2 mW cm⁻²). Photoinitiator solution was added to every hydrogel precursor solution immediately before curing. The volume of photoinitiator solution added to every gel precursor solution was to comprise 10% v/v of the final solution (i.e., a 100 μ L precursor solution would be comprised of 90 μ L of polymer solution and 10 μ L of photoinitiator solution). For all tests, a 5/16 inch diameter circular hole-punch was used to cut out specimens. Before any testing was conducted, equilibrium swelling (overnight incubation in 7.4 pH PBS at 37 °C) was allowed to take place. Hydrogels for all tests were punched out from larger volume (180–660 μ L total volume) bulk gels.

3.4. Evaluation of Network Degradation and Erosion

Network degradation was evaluated by measuring the increased swelling of the hydrogels over time. All swelling measurements were calculated by dividing the wet hydrogel weight by the dry hydrogel weight. Hydro-gels were incubated in 7.4 pH PBS at 37 °C, and this solution was refreshed daily. To eliminate any salt content (i.e., ions from the phosphate buffered saline) from the hydrogels, all hydrogels were incubated in 50 mL of DI water at 37 °C overnight before they were weighed. After wet weight measurements were taken, hydrogels were freeze-dried and weighed again. *N* was equal to 3 for all conditions.

Network erosion was evaluated based on differences in dry weight of the hydrogels. All hydrogels were formed, immediately washed with DI water overnight, freeze-dried, and then weighed. This was taken as the *t* = 0 dry weight of the hydrogels. All hydrogels were then rehydrated, incubated in 7.4 pH PBS at 37 °C that was refreshed daily, and then washed in DI water and freeze-dried again at the desired time points. *N* was equal to 3 in all conditions.

3.5. Mechanical Testing

Compressive testing until failure was performed on the hydrogels to determine their compressive properties. Failure was defined as hydrogel fracture. Measured properties included the compressive modulus (from the linear portion of the stress–strain curve), failure strain, and failure stress (Supporting Information, Equation 1–3). Cross-head speed was set at a constant downward rate of 0.5 mm s⁻¹. For every condition tested, *n* was equal to 3.

Samples were tested on Day 1, and at various incubation times after Day 1 (4, 7, and 14 days) in 7.4 pH PBS that was refreshed daily.

Shear testing was performed to determine the storage and loss moduli of the hydrogels (Supporting Information, Equation 4–6). For all tests, the oscillation of the cross-heads was set at 10 Hz, the normal force applied to the sample was 0.2 N, and strain was increased from 0.01 to 1% by 0.05% increments. Small pieces of paper towel were taped to the cross-heads, and they prevented the sample from slipping as hydrogels adhered strongly to the paper towel interface. For every condition tested, n was equal to 3. All samples were tested after one day of incubation in 7.4 pH PBS.

3.6. Physical Manipulation of Hydrogels

The ability of these gels to undergo physical manipulations in clinical scenarios was tested in three ways: the folding test, the compression test, and the extension test. Images of hydrogels undergoing these three tests and further discussion of the reasoning behind these tests are provided in the Supporting Information, Methods. For every condition, n was equal to 3.

Extension testing was accomplished by grasping the samples on each side with forceps, and then slowly pulling the forceps away from one another. The maximum extension before hydrogel failure was measured. Hydrogels that could withstand 0.05 mm mm^{-1} (5% strain) or greater of extension before failure were considered to have passed this test. We chose this value as a lower limit on the acceptable maximal extension of the hydrogels because nearly any implantation or recovery procedure of these hydrogels in the clinic will, at minimum, involve some small extension of the hydrogels. Additionally, once implanted into the body, nearly any tissue could impart small extensions onto the hydrogel. Similarly, hydrogels had to withstand 1 N of compressive force without fracturing to pass the compression test since nearly any implantation or recovery procedure as well as almost any tissue site would impart small compressive loads onto the hydrogels.

Hydrogels were also tested for their ability to be folded in half and return to their original shape. Fracturing of the hydrogel during folding or an inability to recover their original shape was evaluated as failure. This physical manipulation is indicative of the more complex manipulations that these hydrogels would likely face during a direct implantation.

4. Results

4.1. Characterization of PEG-DTT Reaction

Thiol groups were consumed efficiently during the PEGDA-DTT reaction in aqueous solution. The Measure-iT thiol assay showed a reaction efficiency that ranged from 90 to nearly 100% for all conditions (Supporting Information, Figure S1A). The 3.4 kDa PEGDA reacted at a molar ratio of 0.1 mol DTT to 1 mol PEGDA (hereafter referred to as the DTT:PEG ratio) had the highest reaction efficiency. The reaction efficiency decreased with increasing PEG molecular weight and increasing amounts of DTT. UV-Vis spectroscopy revealed similar trends (Supporting Information, Figure S1B). Absorbance at 283 nm was measured to ensure that thiol moieties were being consumed in a reaction with acrylate

groups and not in disulfide bond formation (data not shown). Increases in absorbance relative to controls at 283 nm were not detected, and there was no apparent emergence of other absorbance peaks within the 220–290 nm region. GPC of the PEG-DTT polymers (described in the Supporting Information, Methods) revealed increasing polymer molecular weight as the DTT:PEG ratio increased and as the PEG molecular weight increased (data not shown), as expected for a step-growth polymerization. NMR of the PEG-DTT polymers also revealed this same trend, and molecular weight data was obtained from this analysis (Figure 2). Additionally, NMR analysis of these polymers revealed decreasing acrylate signal with increasing DTT:PEG ratios, indicating that increasing DTT content in the reaction lead to increased consumption of the acrylate groups (Supporting Information, Figure S2). As a result, we conclude that disulfide bond formation was not occurring, that the thiol groups were being efficiently consumed in a reaction with the acrylate groups, and that the resulting PEG-DTT polymer's molecular weight followed the trends expected in a step-growth polymerization.

4.2. PEG-DTT Hydrogel Degradation and Erosion

The rate of network degradation could be tailored by modulating the amount of DTT, the PEG molecular weight, or the PEG wt%. Over the time frame of 14 days, increasing initial swelling ratio and shorter degradation time frames correlated with increasing DTT content (Figure 3A–C). Additionally, increasing PEG molecular weight correlated with higher equilibrium swelling ratios at $t = 0$ days and shorter degradation times (Figure 3A). When 8 and 12 kDa PEG were used with a 0.8 DTT:PEGDA ratio, the resulting hydrogels underwent complete erosion in less than 24 h, demonstrated by complete absence of a free-standing hydrogel. Adjustments to the PEG concentration used to form the hydrogel (PEG wt%) also resulted in changes to the degradation time frame, with decreasing PEG wt% corresponding with shortened degradation time frames (Figure 3B). In all cases where the swelling ratio increased over time due to network degradation, the dry mass of the hydrogels decreased over time due to network erosion (Figure 3C), as expected for a bulk-eroding network.

4.3. Mechanical Characterization of PEG-DTT Hydrogels

Both the compressive moduli and the failure stress of the hydrogels were modulated by adjusting the PEG molecular weight and the DTT content. In all cases, the compressive moduli decreased with increasing PEG molecular weight and increasing DTT content (Figure 4A). The total range of compressive moduli ranged from 233 to 13 kPa. Notably, the relationship between DTT content and initial compressive modulus was linear in all cases ($R^2 = 0.95$ for all cases for linear regression). The ultimate stress of the PEG-DTT hydrogels followed a similar trend, ranging from 200 to 14 kPa (Figure 4B). Increasing the PEG concentration in hydrogels prepared without DTT (0 DTT:PEG ratio) correlated with an increase in both compressive modulus and ultimate stress, as expected (Figure 5A,B). However, at an 0.6 DTT:PEG ratio, increasing the PEG concentration did not alter the compressive modulus and caused a slight but significant decrease in the ultimate stress of the hydrogels. Thus, while changes to the PEG concentration in hydrogels at a 0 DTT:PEG ratio altered both the compressive modulus and ultimate stress, the same changes in PEG concentration had little effect at higher DTT:PEG ratios.

Both PEG molecular weight and DTT content can be used to control hydrogel shear modulus, consistent with conclusions drawn from compressive testing. Similar to the compressive moduli, the storage moduli decreased with increasing DTT content and PEG molecular weight. This trend was also linear ($R^2 = 0.91$ in all cases for linear regression) (Figure 6). These moduli ranged from 20.6 to 0.7 kPa. The hydrogels did not demonstrate significant viscous behavior, as they displayed low loss moduli (<1 kPa) in all cases (data not shown).

Since the PEG-DTT hydrogels undergo network degradation and erosion over time (see previous section), we hypothesized that their mechanical properties, such as modulus and ultimate stress, would decrease over time as the degradation progressed. Over the course of 14 days, hydrogels without DTT (0 DTT:PEG ratio) showed a decrease (approximately 50%) in both compressive modulus and failure stress (Figure 7A,B). Hydrogels with a 0.4 DTT:PEG ratio showed a linear decrease ($R^2 = 0.97$ for linear regression) in both compressive modulus and ultimate stress over time, which resulted in approximately 75% decreases in both modulus and ultimate stress by Day 14 (Figure 7A,B). The compressive modulus of the 0.6 DTT:PEG ratio condition did not change until Day 14, at which little mechanical integrity remained indicating nearly 100% decrease in the compressive modulus. Its ultimate stress showed an approximately 60% decrease by Day 7 before the loss of mechanical integrity at Day 14 (Figure 7A,B). Ultimate compressive strain showed no statistically significant changes over the course of this experiment for any condition (data not shown). These data demonstrate that while all formulations underwent a decrease in both modulus and failure stress during the course of this experiment, modulating the DTT content of the hydrogels provided a mechanism to control both the total magnitude of and rate of the loss of mechanical properties.

4.4. Physical Manipulation of Hydrogels

Hydrogels were subjected to a series of tests to determine whether they could withstand physical manipulations that are common in biomedical applications. Most of the tested hydrogel formulations could withstand being extended beyond 0.05 mm mm^{-1} (5% strain), and thus passed the extension test (Figure 8A). Hydrogels that did not pass this test fell into two categories. Those with a 0–0.2 DTT:PEG ratio failed because they underwent brittle fracture during extension, and those with a 0.8 DTT:PEG ratio were too soft to maintain their shape under extension. The maximum extension until failure increased with increasing PEG molecular weight and DTT content (Figure 8A). Similar to the extension test, most hydrogel formulations could support 1 N of force without fracture, and thus passed the compression test (Figure 8B). The only formulations that did not pass the test were those made from 12 or 8 kDa PEG having a 0.8 DTT:PEG ratio, as these hydrogels were too soft to support the 1 N weight.

Unlike both the extension and compression tests, many hydrogel formulations did not pass the folding test (Figure 8C). Those that failed fell into two categories: those that underwent brittle fracture during folding, and those that were too soft to recover their original shape after folding. Hydrogels made from low molecular weight PEG or made with a low DTT:PEG ratio failed because they were too brittle to be folded. Other hydrogels made from

higher molecular weight PEG or with higher DTT: PEG ratios failed because they were too soft to regain their shape after folding. A subset of hydrogels passed all physical manipulation tests: they could be folded in half, withstand compression, and withstand extension, and these formulations are shown in Figure 8D. A summary of the performance of all formulations during these physical manipulations is shown in Supporting Information, Table S1.

Outcomes of both the extension and folding physical manipulation tests were next compared with hydrogel equilibrium swelling ratio, compressive modulus, and shear modulus (Figure 9). The 0.8 DTT:PEG ratio hydrogels made from 8 and 12 kDa PEG were excluded from this analysis as they were too soft to pass any of the physical manipulation tests. Both compressive modulus and shear modulus were inversely correlated with hydrogel extension until failure, and hydrogel equilibrium swelling ratio was directly correlated with hydrogel extension until failure (Figure 9A–C). While hydrogel compressive modulus, shear modulus, and equilibrium swelling were correlated with the outcome of the folding test (passing or failing), in some cases, there was significant overlap in these properties between hydrogels that passed this test and those that failed (Figure 9D–F). Thus, we conclude that equilibrium swelling ratio, shear modulus, and compressive modulus are each poor predictors of the outcome of the folding test. In contrast, hydrogel extension until failure was a strong predictor of the outcome of the folding test, as all hydrogels that had an extension until failure of 0.16 mm mm^{-1} or higher passed the folding test (Figure 9G).

5. Discussion

The Michael-type addition reaction proceeded in all tested DTT:PEG ratios and PEG molecular weights (Figure 2, Supporting Information, Figure S1 and S2), and this is in agreement with previous work by Hudalla et al.^[6] Further study of this reaction by Rieger et al., also demonstrated similar trends to those observed here, such as decreasing reaction efficiency with increasing molecular weight of the reacting species.^[23] The PEG-DTT reaction can be modeled as a step-growth polymerization reaction, and the Flory–Carothers equation relates the stoichiometric imbalance of the PEG and DTT molecules in a step-growth polymerization to the number of repeat units in the resulting polymer (degree of polymerization, x_n) (Supporting Information, Figure S2A and Supporting Information, Equation 7).^[6,24] Thus, adjustments to the DTT:PEG ratio vary the molecular weight of the resulting PEG-DTT polymer as well as the number of hydrolytically labile sites within the polymer backbone (Figure 1 and 2, Supporting Information, Figure S3A, and Supporting Information, Equation 7).

Increasing DTT content or PEG molecular weight led to an increase in equilibrium swelling ratio, likely due to an increase in the molecular weight between cross-links (Figure 3). This relationship is consistent with thermodynamic first principles of hydrogel networks^[25] (Supporting Information, Equation 8; Figure 3). Additionally, Metters and Hubbell^[20] developed a model to predict hydrolytic degradation of a PEG hydrogel based on the kinetics of ester hydrolysis in the polymer backbone. This model was developed to predict the degradation of hydrogels formed by the reaction of multi-arm PEG-acrylates with dithiol molecules.^[20] While the PEG-DTT hydrogels we describe in the current study rely on a

slightly different gelation process, the overall hydrogel design is similar. In particular, each of these approaches includes hydrolytically labile sites situated between non-degradable cross-links that do not degrade during the timeframe of the experiments. Thus, this model is broadly comparable to the PEG-DTT hydrogels presented in this work. Therefore, it is not surprising that the results of our current study are consistent with the model described previously by Metters et al. This model predicts that an increasing number of hydrolytically labile sites between cross-links, increasing PEG molecular weight, or increasing water content of the hydrogels will lead to decreased degradation time frames.^[20] Thus, our observed trends for hydrogel degradation are consistent with the Metters et al. model (Supporting Information, Equation 9 and Supporting Information, Figure S3).

Hydrogel shear modulus can be directly related to the molecular weight between crosslinks based on thermodynamic principles^[26] (Supporting Information, Equation 10), and similar relationships are known for hydrogel compressive and tensile moduli.^[21,26] Thermodynamic principles predict that increases in the molecular weight between crosslinks should cause decreases in the corresponding hydrogel shear moduli (Supporting Information, Figure S3E; Supporting Information, Equation 10), and this trend agrees with experimental observations in the current study, as increasing DTT:PEG ratio and increasing PEG molecular weight each caused corresponding decreases in shear and compressive moduli (Figure 4,6). Thermodynamic principles also predict that changes to polymer concentration in the hydrogel can initially cause differences in hydrogel stiffness, but as the molecular weight between crosslinks increases, these differences become negligible (Supporting Information, Figure 3F). Our experimental observations also agree with this predicted trend, as changes to the concentration of the polymer used to make the hydrogels affected the stiffness of hydrogels containing no DTT, but at high DTT:PEG ratios (increased molecular weight between crosslinks) the polymer concentration had no effect on stiffness (Figure 5). It is also noteworthy that our measurements of a negligible loss modulus in all hydrogel formulations are consistent with observations for photo-crosslinked PEG-methacrylate hydrogels.^[19]

As hydrogel network degradation and erosion progressed, the compressive modulus and failure stress of the hydrogels decreased, which is expected in bulk degrading materials. As hydrolytic degradation of a hydrogel progresses, the molecular weight between cross-links increases, and molecular weight between cross-links is inversely related to hydrogel mechanical properties^[26] (Supporting Information, Equation 10). As a result, we expected that as hydrogel degradation progressed, hydrogel mechanical properties (such as the compressive modulus and failure stress) would decrease. This behavior was indeed observed experimentally with increasing DTT content leading to increased loss of both hydrogel compressive modulus and failure stress over time (Figure 7). Notably, a decrease in both modulus and failure stress also occurred in the 0 DTT:PEG control hydrogel by Day 14. It was assumed for this study that the poly(acrylate) crosslinks were relatively stable, but this is not entirely accurate because the esters proximal to the poly(acrylate) crosslinks can be degraded via hydrolysis.^[18] However, this hydrolysis reaction occurs at a much slower rate when compared to linkages designed to be hydrolytically labile, such as ester linkages that are proximal to thio-ethers.^[18] This may explain why both the modulus and failure stress of the 0 DTT:PEG control hydrogels decreased over time but not to the same extent as the DTT containing hydrogels (Figure 7).

PEG-DTT hydrogels were capable of hydrolytic degradation over controllable time frames (Figure 3) and also possessed mechanical properties that could be tailored to achieve a variety of failure stresses and stiffnesses (Figure 4–6). Many of the hydrogel formulations presented here possessed moduli at or near the moduli of multiple soft tissues, such as the lung (measured in guinea pigs at 5–6 kPa), the kidney (measured in swine at 2.5 kPa), the thyroid (measured in humans at 9 kPa), and the liver (measured in humans at 0.64 kPa).^[27] Additionally, some formulations possessed moduli at or near the moduli of multiple stiffer tissues, such as arterial tissue (measured in mice at 90 kPa), skeletal muscle (measured in rats at 100 kPa), cardiac muscle (measured in mice at 20–150 kPa), and spinal cord tissue (measured in humans at 89 kPa).^[27] Therefore, the PEG-DTT hydrogels described here could be optimized to have stiffnesses matching those desired at most implant sites. Matching the stiffness of the hydrogel to that of an implant site tissue may be important in wound sites such as corneal defects, where inappropriate hydrogel mechanical properties may lead to permanent corneal imperfections.^[28] The ability to control the rate of hydrogel degradation and erosion (Figure 3) as well as the rate of loss of mechanical properties over time (Figure 7) is also an important feature of these hydrogels, since these requirements change dramatically from site to site in the body.^[7,17] Additionally, a subset of hydrogel formulations could withstand simple physical manipulations that these hydrogels would likely encounter in biomedical applications (Figure 8). Notably, the outcomes of these manipulation tests indicate that simply having a high modulus not only lowered the ability of the hydrogel to be extended, but also impaired its ability to withstand the more complex manipulation of folding (Figure 9). In this sense, the compressive and shear moduli of hydrogels were a poor predictor of potential practical applicability in biomedical applications.

Hydrogels synthesized previously using functionalized PEG polymers,^[19] PEG-based copolymers,^[29–32] and PEG-peptide copolymers^[33] have shown mechanical properties within the ranges we observed here.^[11,18,19,21,22,29–37] Previously synthesized hydrogels have also demonstrated similar degradation time frames.^[6,12,18,22,32,35] However, the simplicity and adaptability of our approach to generate PEG-DTT hydrogels here may be advantageous in some biomedical applications. In addition, it is noteworthy that previous studies have focused on measuring hydrogel modulus, and our data demonstrate that high modulus does not necessarily correlate with the ability of a hydrogel to withstand physical manipulation. Virtually all in vivo applications of hydrogels involve some form of physical manipulation, either during implantation or once in an in vivo site. Hydrogels are of interest in the clinic for the multiple purposes including tissue scaffolding,^[5,6,32,36,37] drug and cell delivery applications,^[3,4,11,12] and sequestering of soluble growth factors,^[31] but their use is limited because of the extensive material requirements that hydrogels must meet for use in any of these applications. We posit that the adaptability of the hydrogels presented here makes them able to meet many of these requirements, and thus of potential utility in future clinical applications.

6. Conclusion

Herein, we describe PEG-based hydrogels whose mechanical and degradation properties can be systematically tailored to meet the extensive material requirements of clinical use. We

conclude that through adjustments to simple variables (such as the PEG molecular weight, PEG concentration, and the number of hydrolytic sites within the polymer backbone) we are able to tailor both the degradation rate and modulus of these hydrogels. We further assessed these hydrogels by measuring their performance in three physical manipulations (pushing, pulling, and folding), and we conclude that a subset of formulations were able to withstand these manipulations. We plan to further optimize this subset for specific roles within the clinic. Interestingly, the physical manipulation tests also indicate that simply possessing a high modulus does not correlate with the ability to withstand mechanical manipulation. By comparing the outcomes of the physical manipulation tests to traditional characterization data, we conclude that the best predictor of hydrogel performance in complex manipulations (such as folding) is hydrogel extension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors wish to acknowledge the Gopalan Laboratory at UW-Madison for their assistance in the GPC characterization of the PEG-DTT polymers, and the NMR Facility at Madison for their assistance in the NMR characterization of the PEG-DTT polymers. The authors also acknowledge funding from the National Institutes of Health (R01HL093282) and the University of Wisconsin McPherson Eye Research Institute.

References

- Hoffman AS. Adv Drug Deliv Rev. 2002; 54:3. [PubMed: 11755703]
- Jones A, Vaughan D. J Orthop Nurs. 2005; 9:S1.
- Censi R, Martino PD, Vemonden T, Hennink WE. J Controlled Release. 2012; 161:680.
- Cruise GM, Scharp DS, Hubbell JA. Biomaterials. 1998; 19:1287. [PubMed: 9720892]
- Drury JL, Mooney DJ. Biomaterials. 2003; 24:4337. [PubMed: 12922147]
- Hudalla GA, Eng TS, Murphy WL. Biomacromolecules. 2008; 9:842. [PubMed: 18288800]
- Liu G, Shi Z, Kuriger T, Hanton LR, Simpsons J, Moratti SC, Robinson BH, Athanasiadis T, Valentine R, Wormald PJ, Robinson S. Macromol Symp. 2009; 279:151.
- Nguyen KT, West JL. Biomaterials. 2002; 23:4307. [PubMed: 12219820]
- Peppas NA, Huang Y, Torres-Lugo M, Ward JH, Zhang J. Annu Rev Biomed Eng. 2000; 2:9. [PubMed: 11701505]
- Colthurst MJ, Williams RL, Hiscott PS, Grierson I. Biomaterials. 2000; 21:649. [PubMed: 10711963]
- Yun EJ, Yon B, Joo MK, Jeong B. Biomacromolecules. 2012; 13:1106. [PubMed: 22394182]
- Qui Y, Lim JJ, Scott L Jr, Adams RC, Bui HT, Temenoff JS. Acta Biomater. 2010; 7:959. [PubMed: 21056127]
- Hoare TR, Kohane DS. Polymer. 2008; 49:1993.
- Corkhill PH, Hamilton CJ, Tighe BJ. Biomaterials. 1987; 10:3. [PubMed: 2653447]
- van Dijkhuizen-Radersma, R.; Moroni, L.; van Apeldoorn, A.; Zhang, Z.; Grijpma, D. Tissue Engineering. van Blitterswijk, C., et al., editors. Vol. Ch. 7. Academic Press; London, England: 2008. p. 197-222.
- Zhu J. Biomaterials. 2010; 31:4639. [PubMed: 20303169]
- Rosiak JM, Yoshii F. Nucl Instrum Methods Phys Res Sect B. 1999; 151:56.
- Browning MB, Cosgriff-Hernandez E. Biomacromolecules. 2012; 13:779. [PubMed: 22324325]
- Lin-Gibson S, et al. Biomacromolecules. 2004; 5:1280. [PubMed: 15244441]

20. Metters A, Hubbell J. *Biomacromolecules*. 2005; 6:290. [PubMed: 15638532]
21. Temenoff JS, Athanasiou KA, LeBaron RG, Mikos AG. *J Biomed Mater Res*. 2002; 59:429. [PubMed: 11774300]
22. Zawaneh PN, et al. *Proc Natl Acad Sci U S A*. 2010; 107:11014. [PubMed: 20534478]
23. Rieger J, Van Butsele K, Detrembleur C, Jerome R, Jerome C. *Chem Commun*. 2004:274.
24. Cowie, JMG.; Arrighi, V. *Polymers: Chemistry and Physics of Modern Materials*. Vol. Ch.2. CRC Press; Boca Raton, USA: 2008. p. 26-48.
25. Borzacchiello, A.; Ambrosio, L. *Hydrogels: Biological Properties and Applications*. Barbucci, R., editor. Springer; New York, USA: 2009. p. 9-20.
26. Anseth KS, Bowman CN, Brannon-Peppas L. *Biomaterials*. 1996; 17:1647. [PubMed: 8866026]
27. Levental I, Georges PC, Janmey PA. *Soft Matter*. 2007; 3:299.
28. Grinstaff MW. *Biomaterials*. 2007; 28:5205. [PubMed: 17889330]
29. Li S, Vert M. *Macromolecules*. 2003; 36:8008.
30. Suggs LJ, et al. *J Biomater Sci Polym Ed*. 1997; 9:653. [PubMed: 9686333]
31. Yamaguchi N, Chae B, Zhang L, Kiick KL, Furst EM. *Biomacromolecules*. 2005; 6:1931. [PubMed: 16004430]
32. Martens PJ, Bryant SJ, Anseth KS. *Biomacromolecules*. 2002; 4:283. [PubMed: 12625723]
33. Miller JS, Shen CJ, Legant WR, Baranski JD, Blakely BL, Chen CS. *Biomaterials*. 2010; 31:3736. [PubMed: 20138664]
34. Thornton AJ, Alsberg E, Hill EE, Mooney DJ. *J Urol*. 2004; 172:763. [PubMed: 15247778]
35. Lee KY, Alsberg E, Mooney DJ. *J Biomed Mater Res*. 2001; 56:228. [PubMed: 11340593]
36. Gunn JW, Turner SD, Mann BK. *J Biomed Mater Res A*. 2004; 72:91. [PubMed: 15536643]
37. Bryant SJ, Anseth KS. *J Biomater Res*. 2001; 59:619.

Figure 1.

A) Diagram of the PEG-DTT polymer. Reaction of PEGDA and the small molecule dithiothreitol (DTT) produces the PEG-DTT polymer via a Michael-type addition reaction in PBS at 7.4 pH. When the molar ratio of DTT to PEGDA is imbalanced in favor of PEGDA, the PEG-DTT polymers will be predominantly terminated with acrylate groups. B) Degradation mechanism of the PEG-DTT polymer. A hydroxyl ion attacks the carbonyl carbon proximal to the thio-ether, and this yields a carboxylic acid and an alcohol. C) Cross-linking of remaining acrylate groups to form a hydrogel. The acrylate cross-links formed here are expected to be relatively stable at physiologic temperature and pH, but the polymer backbone contains multiple hydrolytically labile sites.

Figure 2.

A) Representative NMR of the acrylate peaks and the repeating unit peaks utilized to estimate polymer molecular weight. Note that the shown spectrum has been cropped (indicated by black dashed bar) to show only the pertinent regions. Additionally, the main repeating unit peak is vertically cropped in order for all other peaks to be visualized here. B) Estimated polymer molecular weights (MW) for PEG-DTT polymers made from 12, 8, and 3.4 kDa PEG (indicated at top). DTT-PEG molar ratios (listed at left in each column) were increased from 0 to 0.8. Note that * indicates that the acrylate signal was so low (due to consumption of acrylate groups in the PEG-DTT reaction) that it could not be accurately resolved from noise in the spectrum.

Figure 3.

Swelling and mass loss over time for PEG-DTT hydrogels. All conditions containing DTT had higher equilibrium swelling ratios than 0 DTT controls, and many conditions had an increase in swelling ratio over time. In all graphs, * indicates statistical significance relative to 0 DTT: PEG controls ($p < 0.05$). ‡The hydrogels had degraded to such an extent that they could not be handled for mass measurement. A) Swelling ratios (wet mass/dry mass) of hydrogels made from 3.4, 8, and 12 kDa PEG with 0, 0.2, 0.4, 0.6, or 0.8 DTT:PEG ratios at a concentration of 15 wt% over a time frame of 14 days. Note that 0.8 DTT:PEG data are not shown for 8 and 12 kDa conditions since complete erosion occurred in <24 h. B) Hydrogels made from 8 kDa PEG with an 0.6 DTT:PEG ratio at varying concentrations of the DTT-PEG polymer (wt%). Lowering the polymer wt% from 15 wt% to 12.5 and 10 wt% caused corresponding decreases in the degradation time frame. Note that the 15 wt% data is replotted from (A). C) Mass loss (dry mass/original dry mass) from 8 kDa, 15 wt% hydrogels containing 0, 0.2, 0.4, and 0.6 DTT:PEG ratios.

Figure 4.

Compressive testing of PEG-DTT hydrogels. In all cases, PEG molecular weight and DTT content were found to inversely correlate with compressive modulus and failure stress. *Statistical significance relative to the 0 DTT controls ($p < 0.05$). ‡The hydrogels were too soft for compressive testing. A) Compressive moduli of hydrogels made from 15 wt% PEG of either 3.4, 8, or 12 kDa molecular weight with varying amounts of DTT. B) Failure stress of hydrogels made from 15 wt% PEG of either 3.4, 8, or 12 kDa molecular weight with varying amounts of DTT.

Figure 5.

Compressive modulus (A) and failure stress (B) of hydrogels made from 10, 12.5, and 15 wt % 8 kDa PEG hydrogels with a DTT:PEG ratio of 0 or 0.6. Compressive modulus and failure stress decreased with decreasing wt% in the 0 DTT condition, but not in the 0.6 DTT:PEG condition. Note that 15 wt% data is replotted here for comparison purposes from Figure 3. *Statistical significance relative to the 15 wt% condition.

Figure 6. Shear modulus of PEG-DTT hydrogels made from 0, 0.2, 0.4, 0.6, and 0.8 DTT:PEG ratios at a polymer concentration of 15 wt%. A) PEG of 3.4, 8, and 12 kDa molecular weights were used. * Statistical significance from the 0 DTT control. † The hydrogel was too soft to be tested.

Figure 7.

PEG-DTT hydrogel compressive moduli and failure stress over time. Hydrogels were made from 8 kDa PEG at a polymer concentration of 15 wt%. Compressive moduli of hydrogels made at 0, 0.4, and 0.6 DTT:PEG ratios are shown over time in (A). Failure stresses of these hydrogels are shown in the same order in (B). In all graphs, * indicates statistical significance from the corresponding condition at Day 1. ‡The hydrogels were too soft for measurement.

Figure 8.

Physical manipulation testing of PEG-DTT hydrogels was carried out with three separate tests. A table summarizing the complete results of all tests is included as Supporting Information Table 1. A) Results of the extension test in 3.4, 8, and 12 kDa PEG hydrogels. The dashed line indicates 0.05 mm mm⁻¹ extension, which we chose as a minimum value to represent the small amounts of extension that these hydrogels would have to undergo in nearly any biomedical application. Extension until failure increased as PEG molecular weight and DTT content increased. *Statistical significance relative to the 0 DTT control ($p < 0.05$). ‡ The hydrogels were too soft to be extended for the measurement. B) Table summarizing the results of the compression test. All formulations passed this test except for the 8 and 12 kDa 0.8 DTT:PEG conditions, which were too soft to support the 1 N weight. C) Table summarizing the results of the folding test. All formulations that failed this test were too brittle to withstand folding, except for the 8 and 12 kDa 0.8 DTT:PEG conditions, which were too soft to recover their original shape after folding. D) Summary of hydrogel formulations that passed all three tests.

Figure 9. Relationships between bulk hydrogel properties and the outcome of physical manipulation tests. A) and B) Normalized compressive modulus and normalized shear modulus, respectively, against normalized hydrogel extension until failure. Compressive and shear moduli are seen to inversely correlate with hydrogel extension. C) Normalized hydrogel swelling ratio against normalized extension until failure. Swelling ratio is seen to directly correlate to normalized hydrogel extension until failure. D–G) Performance in the folding test (pass or fail) plotted against normalized compressive modulus, shear modulus, swelling ratio, and extension, respectively. Clear categories for hydrogels failing and hydrogels passing the folding test are found when compared to normalized extension (G), with all hydrogels that can withstand 0.16 mm mm^{-1} of extension being able to withstand the folding test.