Macromolecules

ARTICLE

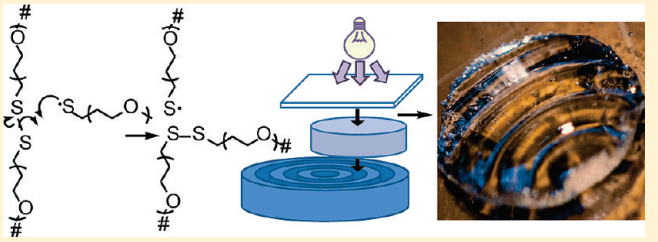
pubs.acs.org/Macromolecules

Photodegradable, Photoadaptable Hydrogels via Radical-Mediated Disulfide Fragmentation Reaction

Benjamin D. Fairbanks,[†,§] Samir P. Singh,[†] Christopher N. Bowman,[†] and Kristi S. Anseth[*,†,‡]

[†]Department of Chemical and Biological Engineering and [‡]Howard Hughes Medical Institute, University of Colorado, Boulder, Colorado 80309-0424, United States

ABSTRACT: Various techniques have been adopted to impart a biological responsiveness to synthetic hydrogels for the delivery of therapeutic agents as well as the study and manipulation of biological processes and tissue development. Such techniques and materials include polyelectrolyte gels that swell and deswell with changes in pH, thermosensitive gels that contract at physiological temperatures, and peptide cross-linked hydrogels that degrade upon peptidolysis by cell-secreted enzymes. Herein we report a unique approach to photochemically deform and degrade disulfide cross-linked hydrogels, mitigating the challenges of light attenuation and low quantum yield, permitting the degradation of hydrogels up to 2 mm thick within 120 s at low light intensities (10 mW/cm^2 at 365 nm). Hydrogels were formed by the oxidation of thiol-functionalized 4-armed poly(ethylene glycol) macromolecules. These disulfide cross-linked hydrogels were then swollen in a lithium acylphosphinate photoinitiator solution. Upon exposure to light, photogenerated radicals initiate multiple fragmentation and disulfide exchange reactions, permitting and promoting photodeformation, photowelding, and photodegradation. This novel, but simple, approach to generate photoadaptable hydrogels portends the study of cellular response to mechanically and topographically dynamic substrates as well as novel encapsulations by the welding of solid substrates. The principles and techniques described herein hold implications for more than hydrogel materials but also for photoadaptable polymers more generally.



INTRODUCTION

Covalently cross-linked hydrogels represent an important class of biomedical materials as tools for localized drug delivery as well as for the encapsulation of cells for manipulating critical cellular functions and/or tissue development. Covalently cross-linked gels are often formed via the reaction of multifunctional and high molecular weight hydrophilic macromolecules, including proteins, polysaccharides, or synthetic polymers such as poly(ethylene glycol) (PEG), through a variety of polymerization reactions including chain growth (meth)acrylate polymerization,[1] Michael addition,[2] azide−alkyne cycloaddition,[3,4] radical thiol−ene photopolymerization,[5] and oxidation of thiols to disulfides.[6−8]

Of particular interest in recent years are hydrogels that exhibit some chemical and/or physical change in response to an external stimulus. By virtue of their dynamic behavior, many such stimuli-responsive hydrogels, sometimes referred to as "smart" hydrogels, have been applied to drug delivery and tissue engineering in attempts to both permit and control mass transport and/or cellular interaction with(in) the material. Various approaches have been adopted to impart a biological responsiveness to synthetic hydrogels including, but not limited to, oxygen-sensitive disulfide polymerization mentioned previously, polyelectrolyte gels that swell and deswell with changes in pH,[9] thermoresponsive gels that form or contract at physiological temperatures,[10] and peptide cross-linked hydrogels that degrade upon peptidolysis by cell-secreted enzymes.[2] Chemistry and applications vary among the different stimuli-responsive materials though most share a general dependence on the biological context in which they are placed; most often it is the biological context that stimulates the material's response. Recently, an innovative approach to stimuli-responsive hydrogels was reported wherein the user, and not the biological context, provides the stimulus that elicits the materials' response.[11−13] These synthetic, polymeric hydrogels were cross-linked with multifunctional monomers containing a photolabile nitrobenzyl ester, which lyses upon photon absorption, providing the user with exogenous control of material degradation and numerous other material properties.

While the development of such materials has allowed researchers to probe cellular behavior under dynamic and discrete modulation, the strongly absorbing nitrobenzene moiety limits the depth to which materials may be degraded and also represents an irreversible change in the structure that prevents conservation of the mechanical properties and/or cross-link density.[12]

To overcome this latter limitation, hydrogels cross-linked with trithiocarbonate-containing monomers have been synthesized that, through addition and fragmentation reactions, reversibly

Received: January 28, 2011
Revised: March 3, 2011
Published: March 16, 2011

healed upon exposure to UV light.[14] Herein, we present an alternative and complementary material system containing photolabile, photoadaptable groups. Specifically, the material was designed to overcome the challenges of light attenuation and low quantum yield, permitting the degradation of hydrogels up to 2 mm thick within 120 s of exposure to relatively low light intensities and long wavelength ultraviolet light (10 mW/cm^2 and 365 nm, respectively). This degradation was achieved by exploiting the disruption of disulfide bonds by reaction with photogenerated carbon- and phosphorus-centered radicals and subsequent disulfide rearrangement by cascading transfer of sulfur radicals.

Disulfide bonds, typically formed by the oxidation of thiols, have been shown previously to be susceptible to radical cleavage by carboxylate and hydroxyl radicals, a mechanism of radical protein denaturation, resulting in thiyl radical products.[15] Observing relaxation in disulfide and tetrasulfide polymers containing elemental sulfur, Tobolsky et al. hypothesized in 1963 that disulfide exchange could be initiated by radicals generated from the thermal cleavage of the S8 sulfur ring.[16] Moreover, addition of certain disulfides to radical chain growth polymerizations has been shown to impart some living polymerization attributes via fragmentation reactions,[17] and radical exchange reactions with disulfides have been employed in the synthesis of tertiary (thiocarbonyl) sulfanyl compounds.[18]

In this article, hydrogels are formed by the oxidation of thiol-functionalized 4-armed poly(ethylene glycol) macromolecules in solution. This disulfide cross-linked hydrogel was swollen in a solution of a lithium acylphosphinate (LAP) photoinitiator,[19] recently demonstrated to possess a higher efficiency and quantum yield than alternative water-soluble cleavage-type radical photoinitiators.[20] Upon exposure to light at a wavelength of 365 nm, the radicals formed from the scission of the photoinitiator attack and cleave the disulfides, liberating thiyl radicals which then undergo fragmentation and exchange with other disulfides. When the concentration of radicals generated by the initiator is on the order of the concentration of sulfur atoms in the network, the network can be completely degraded into soluble PEG segments.

If, however, the number of radicals generated is much less than the number of sulfur atoms in the gel (accomplished by limiting the initial photoinitiator concentration), the network will instead adapt to an applied strain to minimize the free energy of the system.[21−23] Under such conditions, self-healing is possible as disulfide exchange at the interface of two prefabricated gels in contact will chemically anneal to form a single gel of combined dimensions.

This simple approach to photodegradable, photoadaptable hydrogels portends the study of cellular response to mechanically and topographically dynamic substrates as the photoreactions are performed under cytocompatible conditions. One might also envision encapsulation methods by the annealing of preformed solid substrates as an alternative to the typical encapsulation by gelation of liquid solutions. Moreover, the principles and chemistry described herein are not limited to hydrogel materials but may prove beneficial when performing photoinitiated radical reactions in the presence of disulfide-containing materials and the development of photoadaptable polymers more generally.

MATERIALS AND METHODS

Synthesis of Monomer and Initiator. Thiol-terminated four-armed poly(ethylene glycol) PEG4SH was prepared in a manner similar to Goessl et al.,[6] but with some significant modifications. Five grams of hydroxy-terminated, four-armed PEG (MW 10 000) (Jenkem) was dissolved in 70 mL of toluene in a round-bottomed flask and refluxed at 80 °C. Sodium hydride (Sigma-Aldrich), 1.5 mol equiv with respect to hydroxyls, was added to the solution. Allyl bromide (Sigma-Aldrich), 1.5 mol equiv relative to hydroxyls, was diluted with 10 mL of toluene and added dropwise to the solution via an addition funnel. The reaction was refluxed overnight at 80 °C.

Any unreacted sodium hydride was neutralized with the addition of 1 mL of methanol, whereupon sodium salts were removed via filtration, and the PEG was recovered from solution by precipitation in cold diethyl ether. The PEG tetraallyl ether was dried in vacuo and then redissolved in 50 mL of methanol in a 100 mL round-bottom flask, to which was added 0.10 g of the radical photoinitiator 2,2-dimethoxy-1,2-diphenylethan-1-one (trade name Irgacure 651, BASF). Thiol acetic acid (Sigma-Aldrich), 1.5 equiv relative to the allyl groups, was added, and the solution was stirred vigorously under exposure to 10 mW/cm^2 365 nm UV light for 20 min. PEG thioacetate was recovered from solution by precipitation and dried as before. Dried PEG thioacetate was dissolved in 20 mL of deionized water (diH_2O), whereupon 20 mL of 2 M NaOH was added and the solution was stirred for 5 min, and 11 mL of 4 N HCl solution was added to acidify the solution and discourage disulfide formation. The aqueous solution was extracted with equivalent volumes of chloroform twice, combined fractions were concentrated by rotary evaporation, and product was recovered by precipitation in cold diethyl ether. Substitution was determined to be ∼90% by both Ellman's analysis and proton NMR. The initiator LAP was synthesized as described previously.[20]

Gel Formation. Polymerization of the PEG4SH was performed in diH_2O. To a 10 wt % solution of PEG-4SH was added 30% hydrogen peroxide (Sigma-Aldrich) to a final concentration of 1% and sodium iodide (Sigma-Aldrich) to a concentration of 1 mM. The solution was then injected between glass slides separated with silicon rubber gaskets to form circular disks. After at least 3 h, 40 μL gels (height = 1 mm, diameter = 7 mm) were removed from molds and put in 1 mL of diH_2O each. After an hour at room temperature, gels were removed to fresh diH_2O and placed at 4 °C for subsequent experiments. Hydrogels were placed in 1 mL of aqueous LAP solution for 60 min prior to light exposure.

Mechanical and Chemical Characterization. *In situ* dynamic photorheometry was performed on an Ares TA 4400 rheometer with photoexposure attachments as described previously.[5,20] Samples were measured at 20% strain and 20 Hz frequency with parallel-plate geometry. For strained exposures, the rheometer was employed to provide the normal force. Collimated light was supplied by an EXFO Omnicure 1000 at 365 nm and 10 mW/cm^2 for all experiments on and off the rheometer.

Topographical features of hydrogel were imaged with a Leica MZIII stereomicroscope and the dimensions quantitatively examined by profilometry (Stylus Profiler, Dektak 6M, force = 1 mg and radius = 12.5 mm).

^{1}H NMR and ^{31}P NMR were performed on a Bruker AV-III 300 MHz NMR spectrometer in D_2O. All spectra were analyzed with MestreNova 6.1.

RESULTS AND DISCUSSION

An idealized schematic of the oxidation of the PEG-4SH, performed with 1 mM NaI and 1% H_2O_2, to form networks is shown in Figure 1. Following polymerization and removal of the oxidants, degradation behavior of the cross-linked gels in the presence of varying concentrations of the photoinitiator was examined (Figure 2). *In situ* dynamic photorheometry suggests that, upon light exposure and initiator cleavage, both radical

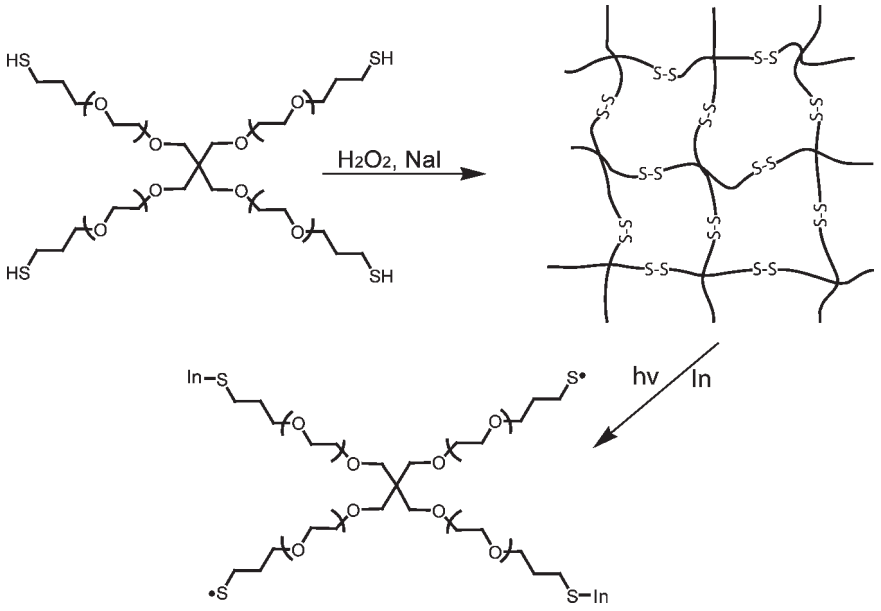


Figure 1. Gels are formed by the oxidation of thiols on a four-armed PEG with 1% H_2O_2 and 1 mM NaI and subsequent degradation by photogenerated radicals (In). Note the degradation products include initiator fragment-terminated chains as well as thiyl radical-terminated chains.

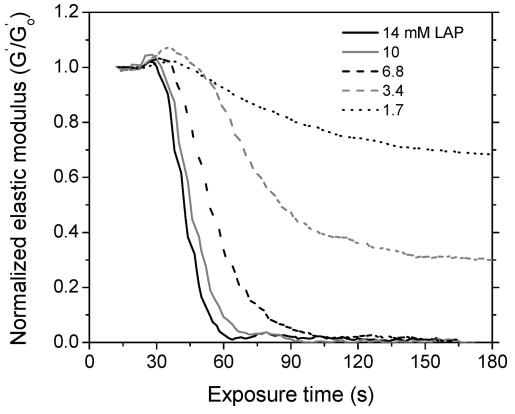


Figure 2. Photorheometry of the normalized elastic modulus as a function of degradation time with varying initiator concentrations. The original shear elastic modulus was 7.2 ± 0.3 kPa.

initiator fragments react with the sulfur atoms in disulfide bonds. Gels infused with varying concentrations of the LAP initiator exhibit concentration dependent degradation behavior as expected. According to the theory of rubber elasticity, the elastic modulus of a material is proportional to the concentration of elastically active cross-links; in this case these would be disulfide bonds that connect one segment of the network to another segment of the network. As seen from the degradation of the hydrogel infused with 1.7 and 3.4 mM of initiator, doubling the concentration of radical generators (and presumably doubling the ultimate number of radicals generated) results in approximately twice the decrease of modulus, from 30% to 70%.

To better understand the degradation behavior and mechanisms, one can first calculate the final concentration, after polymerization and swelling, of sulfur atoms in the hydrogels, which is ~19 mM. Using the Flory−Stockmayer equation,[24,25] the critical conversion at gelation is estimated as 34%, which suggest that 66% of the disulfide bonds must be cleaved to observe reverse gelation, the transition from a viscoelastic solid to liquid. Since thiyl radicals may rapidly recombine to form new cross-links, 66% or 12.5 mM of the sulfur must be bound to initiator fragments for complete degradation of the gel. As seen in Figure 2, initiator concentrations more than half of 12.5 mM result in total dissolution of the network, while those less than half of 12.5 mM do not, suggesting that both radical fragments, resulting from photoinitiator cleavage, participate in the disulfide exchange reaction. This outcome is further supported by ^{1}H NMR and ^{31}P NMR spectra of the degraded products.

Emergence of a triplet proton peaks at 2.7 and 2.5 ppm in the ^{1}H NMR spectrum of a completely degraded hydrogel is consistent with the formation of a trimethylbenzyl thioester (structure shown in Figure 3a) and a phosphonothioic acid ester (Figure 3b), the peaks associated with the protons alpha to the sulfur.

Additionally, ^{31}P NMR of the same sample indicated three distinct phosphorus-containing species at 34, 13.4, and 12.7 ppm (Figure 3). The peak at 13.4 ppm precisely matches the peak of unreacted LAP, while the peak at 34 ppm is indicative of a phosphonothioic acid ester. The peak at 12.7 ppm is consistent with a hypophosphonate which would result from the radical combination of two phosphinate radicals.

The mechanism by which this degradation occurs is shown for the phosphinate fragment of the initiator (Figure 4a). Radical initiator fragments attack disulfides, effectively capping one sulfur while the liberated thiyl radical may undergo additional reactions, including further fragmentation with another disulfide (Figure 4b), termination with another radical initiator fragment, or termination with another thiyl radical to re-form a disulfide (Figure 4c). The process by which the thiyl radical reacts with other disulfides (Figure 4b) is analogous to the RAFT-mediated polymerization[26,27] as well as the addition−fragmentation process that has been shown to occur in allyl sulfide-containing polymer networks.[21,22] This latter process, by which a large number of network strands are broken and replaced with otherwise identical strands, allows the network to adapt to stresses, causing photoinduced plasticity and enabling a range of valuble property and shape changes.

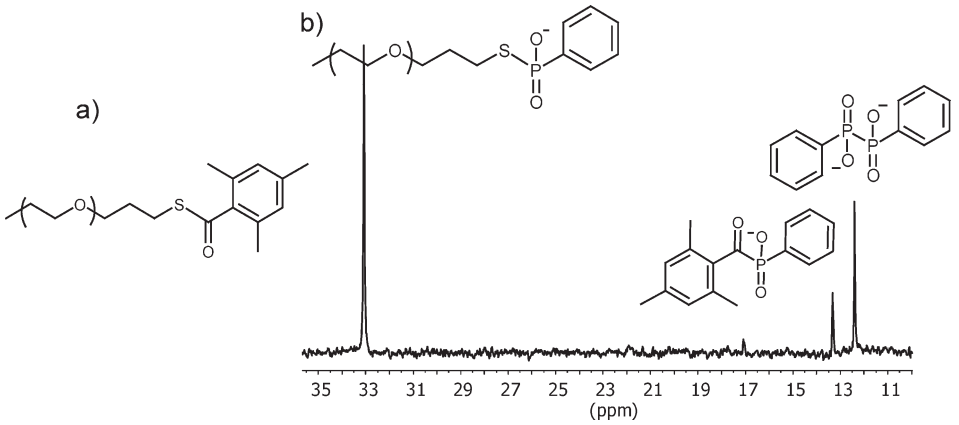


Figure 3. (a) PEG trimethylbenzyl thioester. One possible product from radical degradation of PEG-disulfide networks where the radical initiator fragment reacts with and cleaves the disulfide bridge. (b) ^{31}P NMR spectrum of degraded hydrogel. Peaks are consistent with phosphonothioic acid ester (34 ppm), LAP (13.4 ppm), and hypophosphonate (12.7 ppm) groups.

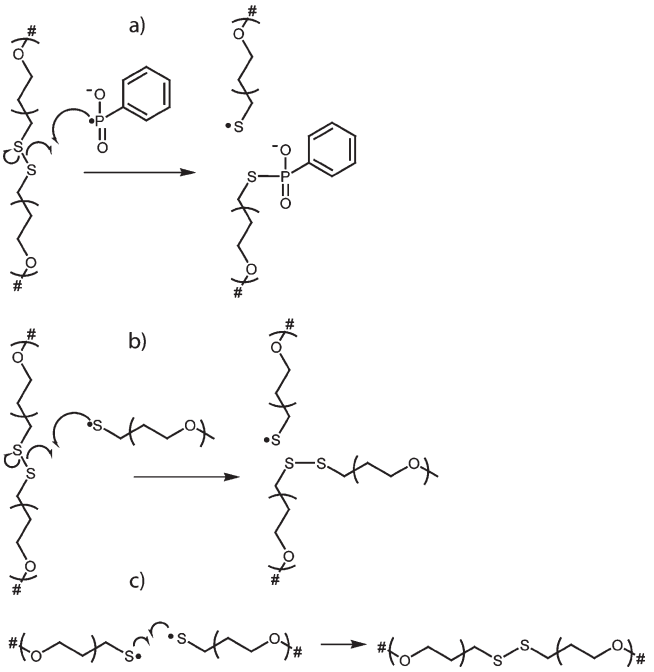


Figure 4. Mechanism of radical degradation and disulfide exchange within PEG hydrogels. Hash marks represent connections to the network. (a) Upon cleavage of the acyl phosphinate, a radical initiator fragment (in this case the phosphonate radical is shown) attacks a disulfide bond, transferring an electron to one of the two sulfurs generating a thiyl radical and a phosphonothioic acid ester-terminated PEG chain. (b) A thiyl radical transfers to another sulfur by fragmentation and degradative chain transfer. The radical disulfide exchange minimizes the free energy of the system, allowing it to accommodate an applied stress on the network. (c) Reversible termination occurs by thiyl radical recombination, re-forming the disulfide bond.

The degradation of the network by an initiator fragment by chain transfer necessarily results in materials with decreased cross-link density as those sulfurs must no longer participate in disulfide bonds. It should be noted, however, that both the benzyl thioester group and phosphonothioic acid ester have been shown to cleave in alkaline and acidic conditions, regenerating a thiol.[28−30] Thus, the radial scission and initiator fragment substitution may be considered a temporary protection of thiol groups. For photodegradable materials, the results presented in Figure 2 indicate that the extent of network degradation can be precisely controlled by the concentration of photoinitiator infused into the gel. This level of control can be used to create photopatterned physical features, either by complete degradation of the network in exposed regions or by incomplete degradation of the network, which, when placed in aqueous buffer, results in increased swelling of the material in exposed regions due to a decreased cross-linking density. Figure 5a shows the scheme by which this procedure was performed using a transparency-based photomask. Noteworthy is that the pattern is transferred to both sides of the hydrogel. Profilometry was used to confirm the presence of the elevated features (broad, exposed lines) and to measure their height, which was ~100 μm. Here, the increase in feature height results from the partial degradation of the cross-linked polymer structure, reducing the cross-linking density and increasing the local equilibrium degree of swelling.

Another way in which topographical features may be created on a disulfide cross-linked hydrogel is by taking an impression of a textured surface and employing photorelaxation for negative pattern transfer. For initiator concentrations well below the cross-link density of the network, the primary reaction will be one of adaptation and rearrangement rather than degradation. If a stress is applied to such a network, during exposure the disulfides will rearrange to minimize the free energy and alleviate the stress. If that stress is accompanied by a strain on the materials, some degree of that strain will be preserved in the regions exposed to light where relaxation and even flow are enabled. Figure 6 illustrates this general principle showing the transfer of a pattern with concentric rings when a prefabricated gel is placed on a template mold and exposed to light. Specifically, Figure 6c is a photograph of a disulfide cross-linked hydrogel, immersed in 0.1 wt % LAP initiator solution, that was placed on top of a surface of raised concentric rings. A normal force corresponding to the weight of two glass microscope slides (5 g) was placed on top of the gel (surface area = 0.50 cm^2), and the gel was exposed to 10 mW/cm^2 light centered at 365 nm. As the disulfide rearranged, the gel adopted the negative of the topographical features against which it was pressed.

Application of a strain followed by photopatterned exposure can also result in the transfer of topographical features via

dx.doi.org/10.1021/ma200202w |*Macromolecules* 2011, 44, 2444–2450

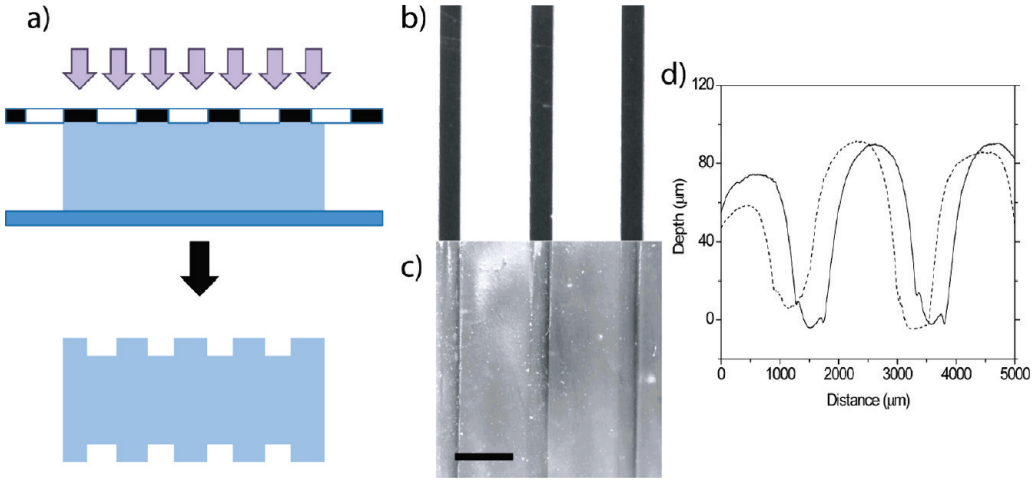


Figure 5. (a) Scheme for photopatterning elevated features by decreasing the cross-linking density in exposed regions, which subsequently swell in aqueous media. (b) Photomask used to pattern the hydrogel. (c) Hydrogel exhibiting elevated features from photopatterning. Scale bar = 1 mm. (d) Profilometry indicating height and breadth of raised features (approximately 100 and 1200 μm, respectively) on both the photomask side (solid line) and the reverse side (dotted line).

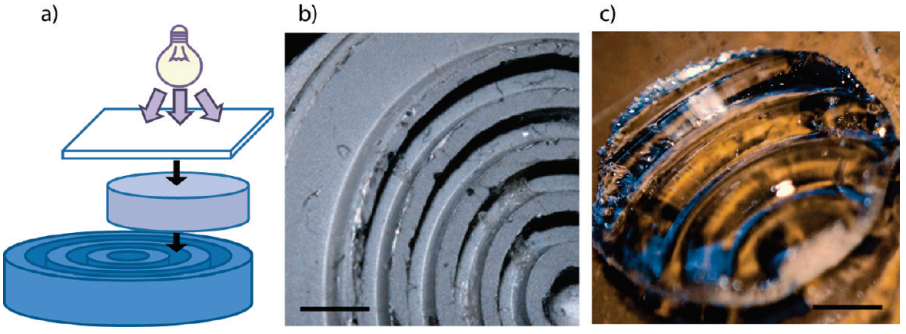


Figure 6. (a) Schematic for the photoimpression patterning of photoadaptable PEG-disulfide hydrogels wherein a LAP-infused hydrogel is pressed against a topographically textured surface and exposed to light. (b) The surface against which the gel was pressed for this demonstration. Scale bar = 1.5 mm. Height of features is ~750 μm. (c) The resulting pattern transferred to a photoadaptable PEG-disulfide hydrogel. Scale bar = 1.5 mm.

network relaxation as well.[23] Figure 7a depicts how this transfer was accomplished. A disulfide cross-linked hydrogel was placed on a flat surface. On top of the hydrogel was placed a photomask (Figure 7b) of transparent squares, 500 μm per side separated by 400 μm. A normal force of 100 g/cm^2, resulting in a deformation of 50% (i.e., the gel was compressed to half its original height), was applied to the hydrogel, whereupon the gel was exposed, through the mask, to 10 mW/cm^2 365 nm light. Where exposed, the gel adapts to the applied strain by breaking and re-forming cross-links in such a way as to minimize the stress. Upon removal of the force that was causing the elastic strain, the unexposed regions recovered their original dimensions while exposed regions remained in their deformed, compressed state as seen in Figure 7. Unlike the features obtained by unstrained photopatterning (Figure 5), the features obtained here are recessed. The depth of the recessed features, determined by profilometry and shown in Figure 7d, was, on average, 90 μm, less than that the 250 μm which would be expected with complete relaxation of exposed regions and 100% recovery of unexposed regions. One likely cause for this discrepancy is the counteraction of increased swelling of exposed regions due to lower cross-linking density.

The photoinduced disulfide rearrangement also enables the healing and welding of hydrogel materials. Two gels that were swollen in 0.1% LAP were pressed together under a transparent plate with a force of 50 g/cm^2 and exposed to light. Upon relief of the pressure the gels were annealed (Figure 8). Similar experiments in the absence of light or radical initiators did not result in any appreciable welding of materials. Mechanical properties of gels prior to and following photohealing were measured by parallel plate rheometry. The elastic modulus of two unhealed gels did not measurably register on the instrument due to slippage between the two gels. Following photohealing, an elastic modulus of 2.2 kPa was measured. This number is consistent with the photorheometry degradation experiments as reported in Figure 2, where full exposure of a gel with 0.1 wt % LAP (or 3.4 mM) results in a 65% decrease in modulus. This shear elastic modulus is an average mechanical property measurement, and while it confirms the photohealing of the hydrogels, it should not be extrapolated as a measurement of the strength of the welded interface.

While the radical cleavage of disulfides by radicals is well established, this research demonstrates that carbon- and phosphorus-centered radical products of photocleavage can effect disulfide lysis for photdegradation and disulfide rearrangement. This outcome is particularly relevant as degradation, shape, and property control are all desirable in a wide range of hydrogel

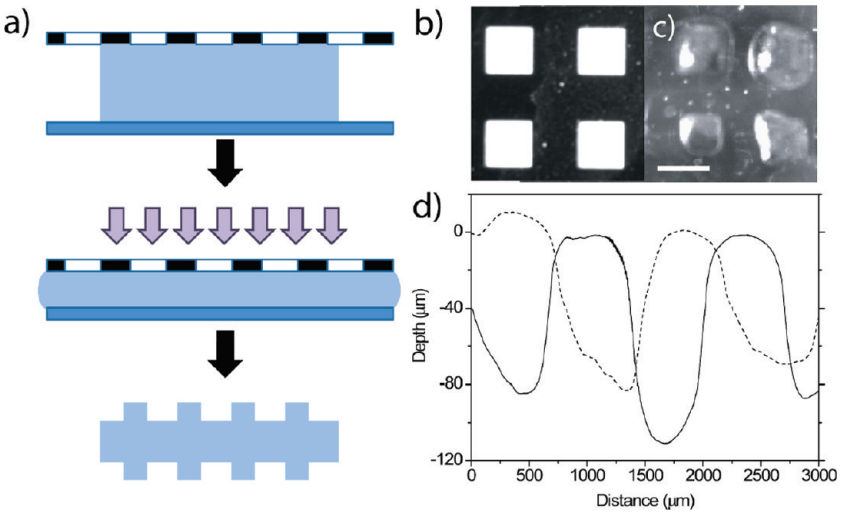


Figure 7. (a) Scheme for creating topographical patterns via strained exposure. A photomask is placed on the gel. The gel is compressed by application of normal stress and exposed to masked UV irradiation. Upon relief of force, the gel exhibits topographical features on both sides due to relaxation in UV-exposed regions. (b) Photomask for patterning hydrogel by method described. White squares are 500 μm separated by 400 μm. (c) Resulting recessed patterns on surface of hydrogel. (d) Profilometry of the surface of the patterned gel. The mask-side profile is the solid line while the reverse side is represented by the dotted line.

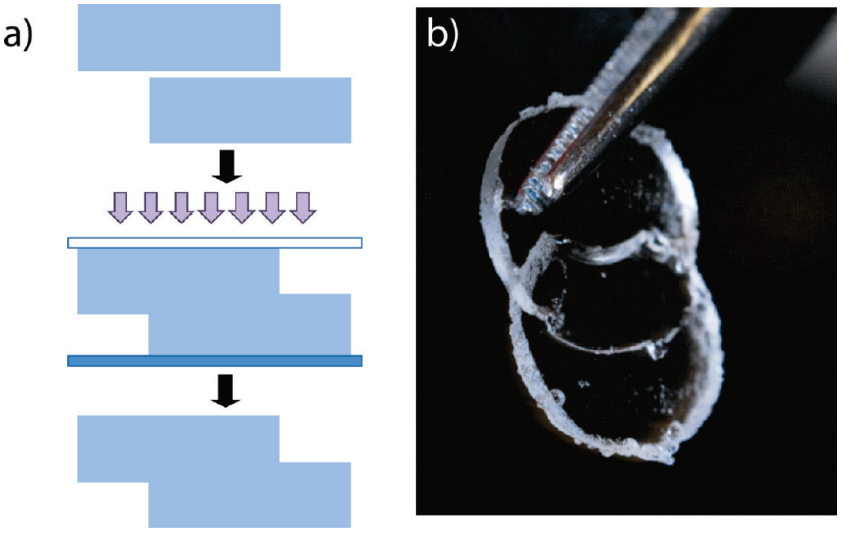


Figure 8. (a) Scheme for photohealing of disulfide hydrogel. Gels are pressed together to ensure adequate contact. Light exposure and accompanying disulfide rearrangement result in the annealing of the two hydrogels. (b) Two hydrogels that have been covalently annealed by photoinitiated disulfide rearrangement.

applications. Disulfide degradation could have substantial potential in the development of stimuli-responsive materials. Many researchers have used disulfides to stabilize self-assembling micro- and nanostructures.[31,32] Among the most widely implemented and cited of these materials are those developed by Stupp and co-workers wherein amphiphilic, cysteine-bearing peptides self-assemble into nanofibers[33] and macroscopic materials such as hydrogels and membranes. The inclusion of radical generators could add to the capabilities of these disulfide-stabilized materials, yielding another dimension of stimulus-responsive character. Disulfides have also been incorporated into synthetic polymers for drug delivery.[8,34,35] Photoinitiated radical degradation and disulfide rearrangement provide a route to exogenous control over the release of therapeutic agents.

Also worth noting is that photoinitiations in the presence of proteins,[36] many of which are held in their ternary conformation by disulfides,[37] are becoming more and more common as researchers explore covalently cross-linked hydrogels for the delivery of therapeutically active enzymes and growth factors. Additionally, disulfide formation is frequently used to create cyclic peptides[29,38] which may be incorporated in tissue engineering constructs for the direction of cellular behavior. These results should not suggest that photoinitiation cannot be used in the presence of peptides and proteins containing disulfide bonds. Polymerization is most often a chain process with a single initiating radical resulting in the formation of many covalent bonds, whereas it is demonstrated herein that the scission of a single disulfide bond and subsequent termination of thiyl radicals

requires one complete initiator molecule. Furthermore, if polymerization occurs much faster than disulfide lysis, such processes can be accomplished with presumably little damage to surrounding proteins and peptides. Researchers should be aware, however, that the disulfide degradation does occur and will presumably occur at a greater rate in the absence of unreacted monomer, such as occurs when polymers are overcured with significant radical generation after the polymerization reaction is otherwise completed.

CONCLUSION

The results presented herein demonstrate the versatility of radical rearrangement of disulfide bonds in hydrogels, but the implications extend to broad areas of polymer and biopolymer engineering. The radical lysis of disulfides and resulting fragmentation reactions of thiyl radicals with disulfides allowed for photodegradation, photoadaptation, and photohealing of hydrogel materials. The application of photoradical generators for the initiation of these processes should expand to the manipulation of various disulfide-containing materials ranging from proteins and peptides to stabilized self-assembled amphiphilic materials to bulk adaptable materials generally.

AUTHOR INFORMATION

Corresponding Author

*E-mail: kristi.anseth@colorado.edu.

Present Addresses

[§]CSIRO Materials Science and Engineering, Clayton, Victoria 3169, Australia.

ACKNOWLEDGMENT

The authors acknowledge funding from the National Science Foundation, grants CBET-0626023 and DMR-1006711. The authors also acknowledge Josh McCall for photographic assistance.

REFERENCES

(1) Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. *Macromolecules* **1993**, *26*, 581–587.
(2) Lutolf, M. P.; Lauer-Fields, J. L.; Schmoekel, H. G.; Metters, A. T.; Weber, F. E.; Fields, G. B.; Hubbell, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5413–5418.
(3) DeForest, C. A.; Polizzotti, B. D.; Anseth, K. S. *Nature Mater.* **2009**, *8*, 659–664.
(4) Polizzotti, B. D.; Fairbanks, B. D.; Anseth, K. S. *Biomacromolecules* **2008**, *9*, 1084–1087.
(5) Fairbanks, B. D.; Schwartz, M. P.; Halevi, A. E.; Nuttelman, C. R.; Bowman, C. N.; Anseth, K. S. *Adv. Mater.* **2009**, *21*, 5005–5010.
(6) Goessl, A.; Tirelli, N.; Hubbell, J. A. *J. Biomater. Sci., Polym. Ed.* **2004**, *15*, 895–904.
(7) Hahn, S. K.; Park, J. K.; Tomimatsu, T.; Shimoboji, T. *Int. J. Biol. Macromol.* **2007**, *40*, 374–380.
(8) Li, C. M.; Madsen, J.; Armes, S. P.; Lewis, A. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 3510–3513.
(9) Zhao, Y.; Kang, J.; Tan, T. W. *Polymer* **2006**, *47*, 7702–7710.
(10) Huang, G.; Gao, J.; Hu, Z. B.; John, J. V. S.; Ponder, B. C.; Moro, D. *J. Controlled Release* **2004**, *94*, 303–311.
(11) Kloxin, A. M.; Kasko, A. M.; Salinas, C. N.; Anseth, K. S. *Science* **2009**, *324*, 59–63.
(12) Kloxin, A. M.; Tibbitt, M. W.; Kasko, A. M.; Fairbairn, J. A.; Anseth, K. S. *Adv. Mater.* **2010**, *22*, 61–66.
(13) Wong, D. Y.; Griffin, D. R.; Reed, J.; Kasko, A. M. *Macromolecules* **2010**, *43*, 2824–2831.
(14) Amamoto, Y.; Kamada, J.; Otsuka, H.; Takahara, A.; Matyjaszewski, K. *Angew. Chem., Int. Ed.* **2011**, *50*, 1660–1663.
(15) Favaudon, V.; Tourbez, H.; Houee-Levin, C.; Lhoste, J. M. *Biochemistry* **1990**, *29*, 10978–10989.
(16) Tobolsky, A. V.; Takahashi, M.; Macknight, W. J. *J. Phys. Chem. A* **1964**, *68*, 787.
(17) Endo, K.; Shiroi, T.; Murata, K. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 145–151.
(18) Thang, S. H.; Chong, Y. K.; Mayadunne, R. T. A.; Moad, G.; Rizzardo, E. *Tetrahedron Lett.* **1999**, *40*, 2435–2438.
(19) Majima, T.; Schnabel, W.; Weber, W. *Macromol. Chem. Phys.* **1991**, *192*, 2307–2315.
(20) Fairbanks, B. D.; Schwartz, M. P.; Bowman, C. N.; Anseth, K. S. *Biomaterials* **2009**, *30*, 6702–6707.
(21) Scott, T. F.; Schneider, A. D.; Cook, W. D.; Bowman, C. N. *Science* **2005**, *308*, 1615–1617.
(22) Kloxin, C. J.; Scott, T. F.; Adzima, B. J.; Bowman, C. N. *Macromolecules* **2010**, *43*, 2643–2653.
(23) Kloxin, C. J.; Scott, T. F.; Park, H. Y.; Bowman, C. N. *Adv. Mater.* **2011**10.1002/adma.201100323.
(24) Stockmayer, W. H. *J. Phys. Chem. A* **1944**, *12*, 125–131.
(25) Odian, G., Ed. *Principles of Polymerization*; Wiley: New York, 1991.
(26) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559–5562.
(27) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379–410.
(28) Daniel, K. A.; Kopff, L. A.; Patterson, E. V. *J. Phys. Org. Chem.* **2008**, *21*, 321–328.
(29) Tulla-Puche, J.; Barany, G. *J. Org. Chem.* **2004**, *69*, 4101–4107.
(30) Yang, Y. C. *Acc. Chem. Res.* **1999**, *32*, 109–115.
(31) Eunju, K.; Dongwoo, K.; Hyuntae, J.; Jiyeong, L.; Somak, P.; Narayanan, S.; Yosep, Y.; Namseok, L.; Chan Gyung, P.; Kimoon, K. *Angew. Chem., Int. Ed.* **2010**, *49*, 4408–4408.
(32) Kim, W.; Thevenot, J.; Ibarboure, E.; Lecommandoux, S.; Chaikof, E. L. *Angew. Chem., Int. Ed.* **2010**, *49*, 4257–4260.
(33) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5133–5138.
(34) Lee, Y.; Mo, H.; Koo, H.; Park, J. Y.; Cho, M. Y.; Jin, G. W.; Park, J. S. *Bioconjugate Chem.* **2007**, *18*, 13–18.
(35) Liu, J. Q.; Liu, H. Y.; Jia, Z. F.; Bulmus, V.; Davis, T. P. *Chem. Commun.* **2008**, 6582–6584.
(36) Lin, C. C.; Metters, A. T. *Adv. Drug Delivery Rev.* **2006**, *58*, 1379–1408.
(37) Wedemeyer, W. J.; Welker, E.; Narayan, M.; Scheraga, H. A. *Biochemistry* **2000**, *39*, 4207–4216.
(38) Wrighton, N. C.; Farrell, F. X.; Chang, R.; Kashyap, A. K.; Barbone, F. P.; Mulcahy, L. S.; Johnson, D. L.; Barrett, R. W.; Jolliffe, L. K.; Dower, W. J. *Science* **1996**, *273*, 458–463.