Mechanical Properties and Degradation of Chain and Step-Polymerized Photodegradable Hydrogels

Mark W. Tibbitt,†‡§ April M. Kloxin,†‡∥ Lisa A. Sawicki,‡∥ and Kristi S. Anseth†‡§∥

†Department of Chemical and Biological Engineering, University of Colorado Boulder, Boulder, Colorado 80303, United States
‡Howard Hughes Medical Institute, University of Colorado Boulder, Boulder, Colorado 80303, United States
§BioFrontiers Institute, University of Colorado Boulder, Boulder, Colorado 80303, United States

ABSTRACT: The relationship between polymeric hydrogel microstructure and macroscopic properties is of specific interest to the materials science and polymer science communities for the rational design of materials for targeted applications. Specifically, research has focused on elucidating the role of network formation and connectivity on mechanical integrity and degradation behavior. Here, we compared the mechanical properties of chain- and step-polymerized, photodegradable hydrogels. Increased ductility, tensile toughness, and shear strain to yield were observed in step-polymerized hydrogels, as compared to the chain-polymerized gels, indicating that increased homogeneity and network cooperativity in the gel backbone improves mechanical integrity. Furthermore, the ability to degrade the hydrogels in a controlled fashion with light was exploited to explore how hydrogel microstructure influences photodegradation and erosion. Here, the decreased network connectivity at the junction points in the step-polymerized gels resulted in more rapid erosion. Finally, a relationship between the reverse gelation threshold and erosion rate was developed for the general class of photodegradable hydrogels. In all, these studies further elucidate the relationship between hydrogel formation and microarchitecture with macroscale behavior to facilitate the future design of polymer networks and degradable hydrogels, as well as photoresponsive materials such as cell culture templates, drug delivery vehicles, responsive coatings, and anisotropic materials.

INTRODUCTION

Covalently cross-linked hydrogels are applied as cell culture templates,¹² absorbent materials, nonfouling coatings,³ contact lenses,⁴ and drug delivery vehicles.⁵ Owing to high water content, reasonable transport of small molecules, and robust mechanical properties, covalently cross-linked hydrogels are particularly attractive materials for a broad array of biological and cellular applications. These reticulated polymer networks are formed by chemical cross-linking of hydrophilic macro-molecules, such as synthetically derived poly(ethylene glycol) (PEG) or poly(vinyl alcohol) and naturally derived hyaluronic acid, gelatin, or alginate, often mildly and in the presence of (PEG) or poly(vinyl alcohol) and naturally derived hyaluronic molecules, such as synthetically derived poly(ethylene glycol) are formed by chemical cross-linking of hydrophilic macro-molecules. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the step-polymerization of complementary, end-terminated comonomers.

Recent work has focused on the formation of cross-linked hydrogels with more ideal and homogeneous microstructures to improve network cooperativity and increase hydrogel mechanical integrity.¹⁵−¹⁸ This has been achieved through the step polymerization of complementary, end-terminated comonomers. Originally, Hubbell and co-workers demonstrated the formation of step-polymerized hydrogels by cross-linking thiol and electron-poor, vinyl functionalized PEG-based molecules for drug delivery and cell encapsulation.¹⁹,²⁰ This paradigm has been extended to fabricate gels utilizing several different step
growth reactions and associated functional groups, including the copper-catalyzed, Huisgen azide–alkyne coupling of functionalized PEG-based comonomers,16,21,22 the coupling of propylamine terminated PEG with succinimidyl glutarate terminated PEG,15 and the photoinitiated thiol–ene coupling of norbornene functionalized PEG with diethil peptides.25

Uniquely, Deforest et al. demonstrated the formation of step-polymerized hydrogels through the copper-free, strain promoted azide alkyne cycloaddition (SPAAC), forming hydrogels in a bio-orthogonal and cytocompatible manner.24

Seminal mechanical analyses of step-polymerized gels have found that these networks possess increased tensile extension16,18 as compared to chain-polymerized analogues, while SANS data have demonstrated that these networks, although not still perfectly ideal, possess fewer heterogeneities in the network microstructure.17 While differences between chain and step polymerization mechanisms and resultant hydrogels are clear, there is little literature on the direct comparison of mechanics and degradation between chain-polymerized and step-polymerized hydrogels. One can gain valuable insight of the structure--function relationship of hydrogels through direct comparisons between chain- and step-polymerized hydrogels with similar chemical structures but profoundly different network connectivities, which will enable the rational design and application of unique hydrogel-based materials.

Furthermore, there is a growing interest in controlling the material properties of both step- and chain-polymerized hydrogels dynamically and in a user-defined fashion using cleavable chemistries whose degradation can be triggered exogenously. Toward this end, recent work has presented a class of photodegradable hydrogels whose physical and chemical properties can be modified by light postfabrication with full spatial and temporal control.21,25−29 Photodegradable hydrogels are appropriate for a myriad of applications in the biomedical and materials sciences. Within the tissue engineering field there is a particular interest in designing cyto-compatible, photodegradable hydrogels that allow the experimenter to control the extracellular microenvironment in the presence of cells in 3D and in real time.29−33 Meanwhile, the drug delivery community is exploiting photodegradable hydrogels to release factors at specific locations and at precise times.34,35 For photodegradable hydrogels to be utilized most effectively in the broad range of applications, a precise and predictable understanding of how irradiation and network structure influence degradation-induced changes in material properties is required. In addition, photodegradation suggests unique opportunities to perform experiments that might provide a better understanding as to how network structure influences material properties during temporally regulated changes to the hydrogel structure.

This work presents the synthesis and characterization of hydrogel networks that are formed by both chain and step polymerizations of a single photodegradable PEG-based macromolecular precursor as model systems to understand differences in both mechanical properties and degradation between the resultant network structures. The formation and associated material properties of the hydrogels are investigated and compared. Furthermore, the photolabile linker in the hydrogel is employed to compare and contrast the photodegradation-induced changes in the two gels. A previously developed statistical-kinetic model of photodegradation is adapted and expanded to describe the degradation of step growth networks. This model accurately describes degradation differences between hydrogels formed by chain and step growth mechanisms, elucidating aspects of the structure–function relationship in hydrogel photodegradation. In all, the material chemistry enables a more robust understanding of how network connectivity and gel architecture influence properties and degradation, and this fundamental understanding should translate into an improved design of hydrogel cells carriers and drug delivery vehicles for biomedical applications.

**MATERIALS AND METHODS**

All reagents were purchased from Sigma-Aldrich and used as received except as otherwise noted.

**Synthesis of Gel-Forming Monomers.** A photolabile, acrylate functionalized monomer, poly(ethylene glycol) diphotodegradable acrylate (PEGdiPDA), was synthesized according to previous published protocols.25−27 Briefly, an acrylated, α-nitrobenzyl ether was synthesized and coupled to poly(ethylene glycol) Bisamine (Mn ∼ 3400 Da; Laysan Bio Inc.) to generate a photoresponsive monomer that is capable of forming both chain- and step-polymerized networks. Four-arm poly(ethylene glycol) macromolecules (Mn ∼ 10 kDa and Mn ∼ 5 kDa; JenKen Technology USA) functionalized with thiol end groups (PEG4SH) were synthesized according to a previously published protocol.30

**Fabrication of Chain-Polymerized Hydrogels.** Chain-polymerized hydrogels were fabricated by copolymerizing PEGdiPDA with monoacrylated poly(ethylene glycol) (PEG; Monomer-Polymer Dajac Laboratories) via redox-initiated, free-radical chain polymerization. Stock solutions of the gel-forming precursors were prepared: 49 mM PEGdiPDA in PBS, 1 M PEGA in PBS, 2 M ammonium persulfate (APS) in PBS, and 2 M triethanolamine (TEOHA) (TEMED) in PBS. Three chain-polymerized hydrogels were fabricated for this work by varying the ratio of PEGdiPDA to PEGA at a constant total polymer wt % of 15 wt %. PEGdiPDA and PEGA were combined in PBS at final solution concentrations of 26.5 mM and 105 mM, respectively to form gel a. PEGdiPDA and PEGA were combined in PBS at final solution concentrations of 17.2 mM and 200 mM, respectively to form gel b. PEGdiPDA and PEGA were combined in PBS at final solution concentrations of 12.3 mM and 250 mM, respectively to form gel c. To initiate polymerization, APS and then TEMED were added to each solution while vortexing at final solution concentrations of 0.2 and 0.1 M, respectively. The solutions were reacted for ∼7 min to achieve complete polymerization, upon which the gels were swelled in PBS. Gels were formed in situ on a parallel-plate shear rheometer (50 μm thick; TA Instruments Ares 4400) or between glass slides separated by 0.5−1.5 mm thick silicon rubber gaskets.

**Fabrication of Step-Polymerized Hydrogels.** Step-polymerized hydrogels were fabricated by copolymerizing PEGdiPDA with thiol-functionalized, four-arm poly(ethylene glycol) (PEG4SH; Mn ∼ 5 k or 10k) via base-catalyzed, Michael-addition. Stock solutions of the gel-forming precursors were prepared: 49 mM PEGdiPDA in PBS pH 8.0, 20 mM PEG4SH 10k in PBS pH 8.0, 40 mM PEG4SH 5k in PBS pH 8.0, and 1 M triethanolamine (TEOHA) in PBS pH 8.0. Three step-polymerized hydrogels were fabricated for this work by varying the molecular weight of the PEG4SH (5K or 10K) and altering the ratio of acrylates to thiols at a constant total polymer wt % of 10 wt %. PEGdiPDA and PEG4SH 10k were combined in PBS pH 8.0 at final solution concentrations of 11 mM and 5.5 mM (r = 1), respectively to form gel d. PEGdiPDA and PEG4SH 10k were combined in PBS pH 8.0 at final solution concentrations of 9.8 mM and 6.0 mM (r = 0.83), respectively to form gel e. PEGdiPDA and PEG4SH 5k were combined in PBS pH 8.0 at final solution concentrations of 15.2 mM and 7.6 mM (r = 1), respectively to form gel f. To accelerate polymerization, TEOHA was added to each solution while vortexing at a final solution concentration of 0.3 M.36 The solution were reacted for ∼25 min to achieve complete polymerization, upon which the gels were swelled in PBS. Gels were formed in situ on a parallel-plate shear rheometer (50 μm thick; TA Instruments Ares 4400) or between glass slides separated by 0.5−1.5 mm thick silicon rubber gaskets.
Modulus Measurements of Hydrogels. In situ polymerization was quantified with time sweep tests on gelling solutions in a parallel-plate shear rheometer (TA Instruments Ares 4400; 8.0 mm diameter and 0.05 mm height). Time sweep tests were conducted at 10 rad/s with 10% strain, which was determined to be in the linear viscoelastic regime for both chain- and step-polymerized hydrogels. Polymerization was followed until the shear storage modulus ($G'$) reached a plateau ($n = 3$ for each gel type). Young’s modulus was reported as three times the shear storage modulus based on the poisson ratio for PEG-based hydrogels.

Swelling Ratio Measurements of Hydrogels. For each gel type, gel samples ($n = 6$) were swollen and weighed in the equilibrium swollen state. The gels were subsequently lyophilized to remove the water weight from the samples and the dry weight was measured. The ratio of the equilibrium swollen weight to the dry weight was used to calculate $q$, the mass swelling ratio. The volumetric swelling ratio, $Q_v$, was then calculated from the mass swelling ratio.

Tensile Testing of Hydrogels. Tensile testing of chain- and step-polymerized hydrogels ($n = 3$ for each gel type) was performed in uniaxial extension with a materials tester (MTS Synergy 100) with a 10 N load head. Swollen hydrogels were cut into ~5 mm × ~25 mm rectangles, and the width, length, and thickness of each sample was measured with digital calipers prior to analysis. Each sample was fixed on the materials tester by compression clamps at the top and bottom of the sample (~5 mm from each end of the gel), and the local environment was kept humidified during the analyses. The initial separation distance was measured with digital calipers, and a constant strain rate of 0.15 mm/mm/min was applied to the sample to failure. The load, stress, strain, and elongation values recorded were used to calculate the stress and strain from the measured dimensions of each sample. The percent strain at failure was calculated as the final extension divided by the initial separation distance multiplied by 100, and the toughness was calculated by numerically integrating for the strain curve.

Degradation of Hydrogels. The kinetics of the photodegradation reaction in both chain- and step-polymerized hydrogels was quantified by irradiating ($\lambda = 365$ nm; $I_0 = 20$ mW/cm$^2$) in situ polymerized gels on a parallel-plate shear rheometer (TA Instruments Ares 4400) and following the modulus evolution as a function of irradiation time. The normalized modulus $G'/G_0$ is proportional to the normalized number density of elastically active network strands $\nu/\nu_0$, where $\nu$ is the number density of elastically active network strands, for each gel system. As irradiation cleaves bonds within the NBE moiety in the PEGdiPDA molecule, elastically active network strands are broken and are polymerized (CP) hydrogels were formed by reacting the tetrafunctional PEGdiPDA with a difunctional comonomer, PEGA, under redox-initiated free-radical chain polymerization. Step-polymerized (SP) hydrogels were formed by reacting the difunctional PEGdiPDA with a tetrafunctional comonomer, PEG4SH, through a base-catalyzed Michael addition. In each case, the network formation occurred through the chemical bonding of the acrylate-functionalized PEGdiPDA. In the chain polymerization each acrylate is difunctional allowing the PEGdiPDA to serve as a tetrafunctional cross-linker, whereas in the step polymerization each acrylate is monofunctional extending the elastically active chains between the tetrafunctional PEG4SH cross-linkers.

Erosion of Channels into Hydrogel Surfaces. Photopatterns (400 µm wide black lines spaced by 400 µm) were originally drawn in Adobe Illustrator and printed on Mylar (Advance Reproductions, North Andover, MA). The photopatterns were attached to glass slides with double-sided tape. Swollen chain- and step-polymerized gels (10 mm × 10 mm × 1 mm) were aligned under the channel patterns and surrounded by PBS to maintain hydration and facilitate dissolution of degraded products during patterning. The gels were then exposed to collimated 365 nm light at 10 mW/cm$^2$ for up to 30 min (Omnicure S1000 with 365 nm filter, liquid filled light guide, and collimating lens, EXFO). Depth of the patterned channels were verified with a profilometer (Stylus Profiler, Dektak 6M).

Model predictions. A statistical-kinetic model of photodegradation in chain-polymerized networks was applied to model the erosion depth as a function of time for the chain-polymerized hydrogels in this work. This model was extended to describe photodegradation in step growth networks by altering the statistical assumptions of network connectivity to account for the differences in network structure. Furthermore, as the time scale of erosion is much faster for step-polymerized hydrogels than chain-polymerized an additional dissolution assumption was included. Briefly, this states that eroded products at the surface of the gel do not instantly diffuse out of the light path, but diffuse through the PBS solution in the light path to a solution sink at the original surface of the gel. By including this simple assumption, the statistical-kinetic model was able to describe the erosion depth as a function of irradiation time in both chain- and step-polymerized hydrogels.

Statistics. All data is reported as mean ± s.e.m.

RESULTS AND DISCUSSION

Formation of Chain- and Step-Polymerized Photodegradable Hydrogels. Photodegradable hydrogels were synthesized via chain- and step polymerization. Chain-polymerized (CP) hydrogels were formed by reacting the tetrafunctional PEGdiPDA with a difunctional comonomer, PEGA, under redox-initiated free-radical chain polymerization. Step-polymerized (SP) hydrogels were formed by reacting the difunctional PEGdiPDA with a tetrafunctional comonomer, PEG4SH, through a base-catalyzed Michael addition. In each case, the network formation occurred through the chemical bonding of the acrylate-functionalized PEGdiPDA. In the chain polymerization each acrylate is difunctional allowing the PEGdiPDA to serve as a tetrafunctional cross-linker, whereas in the step polymerization each acrylate is monofunctional extending the elastically active chains between the tetrafunctional PEG4SH cross-linkers.

Previous studies have shown that the network microstructure of PEG gels formed by chain polymerization is comprised of dense polyacrylate kinetic chains connected by PEG cross-links. These heterogeneities exist on the length scale of the PEG cross-linker, while further heterogeneities form as radical initiation stochastically leads to regions of increased cross-linking density on the micrometer scale. In contrast, PEG
hydrogels formed by step polymerizations have been shown to possess fewer heterogeneities on all length scales. These heterogeneities are limited generally to cyclization and dangling ends. In this manner, the chain polymerization (CP) of PEGdipDA formed a heterogeneous network structure, while the step polymerization (SP) formed a more ideal network. PEGdipDA formed a heterogeneous network structure, while polymerization (CP) of PEGdipDA with PEGA via free-radical polymerization, resulting in a heterogeneous network structure. Step-polymerized hydrogels (SP gels) were fabricated through the copolymerization of PEGdipDA with PEG4SH via Michael-addition polymerization.

Table 1. Physical measurements of Chain- and Step-Polymerized Hydrogels

| gel formulation | gel characterization |
|-----------------|----------------------|
| chain PEGdipDA (mM) | PEGA (mM) | polymer wt % | E (kPa) | Q | tensile strain to failure (%) | tensile toughness (kPa) | shear strain to yield (%) |
| a | 26.5 | 105 | 15 | 19.7 ± 1.5 | 11.5 ± 0.2 | 33 ± 4 | 2.2 ± 0.2 | 89 ± 6 |
| b | 17.2 | 200 | 15 | 19.5 ± 0.7 | 18.0 ± 1.5 | 33 ± 5 | 1.3 ± 0.3 | 130 ± 1 |
| c | 12.3 | 250 | 15 | 17.5 ± 1.8 | 14.1 ± 0.1 | 20 ± 3 | 0.5 ± 0.2 | 93 ± 4 |

| step PEGdipDA (mM) | PEG4SH (mM; Da) | polymer wt % | E (kPa) | Q | tensile strain to failure (%) | tensile toughness (kPa) | shear strain to yield (%) |
|-------------------|----------------|-----------------|-------------|-----|--------------------------|------------------------|------------------------|
| d | 11.0 | 5.5; 10K | 10 | 14.3 ± 1.5 | 20.0 ± 0.8 | 129 ± 11 | 4.1 ± 0.2 | 420 ± 40 |
| e | 9.8 | 6.0; 10K | 10 | 14.8 ± 2.8 | 18.1 ± 0.1 | 87 ± 15 | 6.0 ± 1.4 | 500 ± 70 |
| f | 15.2 | 7.6; 5K | 10 | 10.1 ± 2.5 | 14.5 ± 0.3 | 112 ± 6 | 14.5 ± 2.0 | 290 ± 70 |

The formulations for chain-polymerized (a–c) and step-polymerized (d–f) hydrogels are detailed in the Materials and Methods section. PEG4SH is presented as concentration (mM) and molecular weight (Mw, in daltons).

Mechanical Analysis of Chain- and Step-Polymerized Hydrogels. It has been suggested that the increased homogeneity and network cooperativity of SP hydrogels results in an increase in mechanical integrity, specifically tensile strain to break, as compared to CP hydrogels. Here, network cooperativity is used to describe the ability of multiple network chains within a gel to distribute mechanical stress cooperatively over the network chains. To compare the tensile properties of the chain- and step-polymerized PEG hydrogels studied in this work, tensile testing was conducted on all gels. The percent strains to failure for SP gels were 129 ± 11%, 87 ± 15%, and 112 ± 6% for d, e, and f, respectively (Table 1; Figure 2a,b). These data indicated that, in all cases, the SP gels were more ductile than the CP gels. Further analysis of the tensile testing data revealed that SP gels possessed increased tensile toughness compared to CP gels.
CP gels (Table 1; Figure 2a,c). Specifically, the tensile toughness of the SP gels were 4.1 ± 0.2, 6.0 ± 1.4, and 14.5 ± 2.0 kPa for d, e, and f, respectively, while the tensile toughness for the CP gels were 2.2 ± 0.2, 1.3 ± 0.3, and 0.5 ± 0.2 kPa for a, b, and c, respectively. In addition to the tensile testing, strain sweeps on in situ polymerized hydrogels were conducted to investigate the shear strain to yield for each sample. The SP gels exhibited increased shear strain to yield in all cases as compared to the CP gels (Table 1; Figure 2d), 420 ± 50, 500 ± 70, and 290 ± 70% for SP gels d, e, and f, respectively, and 89 ± 6, 130 ± 1, and 93 ± 4% for CP gels, a, b, and c, respectively.

In both the tensile and shear analyses, it was observed that mechanical integrity was improved for hydrogels formed by step polymerization as compared to chain polymerization. These differences in material properties were conferred by the network structure, specifically the increased network cooperativity and decreased heterogeneity in the SP hydrogel, and suggest that applications that require more ductile or tough materials should employ SP hydrogels. In addition to mechanical integrity, network connectivity directly relates to the diffusion of macromolecules through the hydrogel network and ideal gels should facilitate more uniform diffusion as compared to heterogeneous gels. Finally, these data suggest
that mechanical stresses were translated anisotropically in heterogeneous, CP gels, which may be important for mechanical stimulation or differentiation of mammalian cells.

Photodegradation of Chain- and Step-Polymerized Hydrogels. The CP and SP gels were formed from the same photolabile monomer, PEGdiPDA, rendering them photodegradable. The degradation is facilitated by the o-nitrobenzyl ether (NBE) moieties that reside within the PEGdiPDA monomer (Figure 1) and undergo an irreversible cleavage in the presence of light (one-photon, \( \lambda = 320-436 \) nm; two-photon, \( \lambda = 740 \) nm).\(^{27}\) On account of this property, light was able to cleave bonds within the materials, resulting in the breakage of elastically active network strands and, ultimately, erosion of the gel with light exposure (Figure 3a,b). For the analysis of photodegradation in chain- and step-polymerized hydrogels, a representative CP gel (formulation b) and a representative SP gel (formulation d) were analyzed and compared. Prior to erosion, photodegradation led to an exponential decrease in the shear storage modulus (Figure 4 compared. Prior to erosion, photodegradation led to an exponential decrease in the shear storage modulus (Figure 4).

To investigate how network structure influences mass loss and erosion rates of the CP and SP gels, physical channels were eroded into the surfaces of both gels. While rheometry results indicated that the inherent rate of photodegradation is independent of network structure, the erosion rates for the representative CP and SP gels diverged even at short time scales (Figure 4a). Statistical-kinetic models of photodegradation and erosion in chain-polymerized\(^{27}\) and step-polymerized hydrogels were applied to describe the depth of channel formation as a function of time to elucidate how network connectivity leads to dramatic differences in pattern formation rate. In both cases, the simple statistical-kinetic model captured the observed erosion behavior (Figure 4a), which indicates that the statistical-kinetic model includes the relevant physics of erosion in CP and SP photodegradable gels. These results demonstrate that the lower network connectivity observed in SP gels leads to an increased rate of erosion. For these experiments, the assumption of dissolution of erosion by-products was accounted for in the rapidly degrading step-polymerized gels (see Materials and Methods).

In both of these models, the critical parameter that dictates the erosion rate is the critical fraction of cleaved NBE species, \( P_{rg} \), which governs reverse gelation. Here, reverse gelation refers to the critical extent of bonds cleaved that causes the insoluble gel to erode completely into soluble polymer chains (Figure 3a,b). The network structure of the representative CP gel (formulation b) resulted in a \( P_{rg} = 0.42 \) while the representative CP gel (formulation d) resulted in a \( P_{rg} = 0.77 \). A critical time scale, \( t_c \), was defined as the time to reach reverse gelation at the surface of a photodegradable hydrogel and is a function of \( P_{rg} \):

\[
 t_c = \frac{-\ln(1 - P_{rg})}{k_{eff}I_0} 
\]

where, \( k_{eff} \) is the effective kinetic constant of cleavage of the NBE moiety; \( I_0 \) is the intensity of the incident irradiation. Since the cleavage reaction followed first-order kinetics with the same effective kinetic constant in both gels and each was exposed to the same incident irradiation, the difference in \( P_{rg} \) alone determined the difference in erosion time constants, \( t_c \) = 490 s for the CP gel and \( t_c \) = 180 s for SP gel.

The critical erosion time scale, \( t_c \), governed not only the time to erode the surface of the gel, but also the rate at which erosion progresses through the depth of the gel. A critical length scale, \( z_c \), was defined from the Beer-Lamber Law:

\[
 z_c = \frac{1}{2.3 \varepsilon C_i} 
\]

Here \( \varepsilon \) is the molar absorbitivity of the NBE moiety; \( C_i \) is the concentration of the NBE moiety. A rate for which the erosion progressed through the gel was calculated as the critical length scale of photodegradation divided by the critical time scale of photodegradation:

\[
 z_c \frac{t_c}{t_i} = \frac{-k_{eff}I_0}{2.3 \varepsilon C_i \ln(1 - P_{rg})} 
\]

Owing to the differences in the \( P_{rg} \) and the concentration of NBE moieties in the CP and SP gels, the rate of erosion was significantly faster for the SP gel as compared to the CP gel. The simple scaling analysis predicted an erosion rate of 3.6 and 18.4 \( \mu m/min \) compared to experimental values of 4.4 ± 0.1 and 18.6 ± 2.0 \( \mu m/min \) for the CP and SP gels, respectively.

The above analysis of the relationship between erosion rate and \( P_{rg} \) holds for the specific chain-polymerized and step-polymerized hydrogels in this manuscript as well as for gels formed with the same network connectivity, i.e., the same \( P_{rg} \). However, more broadly, the equations hold for the general class of photodegradable hydrogels for which the network structure and physical parameters are known. Specifically, step-polymerized gels have been formed from PEG monomers with varying functionality leading to different network connectivity.\(^{38,40}\) For instance, the cross-linking of an octa-
ional, thiol-terminated PEG with a tetrafunctional, vinyl−sulfone-terminated PEG would form a network with different connectivity than a tetrafunctional, thiol-terminated PEG and a trifunctional, vinyl−sulfone terminated PEG. Differences in network connectivity are directly related to $P_{rg}$ and, ultimately, the rate of erosion. The reverse gelation point for step-polymerized hydrogels, formed from two complementary monomers, has been adapted from classical derivations by Flory and Rehner that describe network formation in step growth polymerizations:23,41,42

$$P_{rg}^{step} = 1 - \frac{1}{\sqrt{(f_A - 1)(f_B - 1)}}$$ (4)

where, $f_A$ is the functionality of the A-terminated monomer; $f_B$ is the functionality of the B-terminated monomer; and $r$ is the stoichiometric ratio of A to B. This derivation based on the Flory−Rehner theory assumes complete reaction of all functional end groups in the polymer network without loops, dangling ends, or entanglements. Therefore, real systems, such as the SP gels in this work, will have an effective $P_{rg}$ lower than the ideal calculation as loops, dangling ends, and entanglements form during polymerization. The reverse gelation point for chain-polymerized hydrogels has been adapted from classical derivations of Macosko and Miller:43–45

$$P_{rg}^{chain} = 1 - \frac{1}{\sqrt{N - 1}}$$ (5)

where, $N$ is the number of cross-linking molecules per polycrylate kinetic chain, which is determined by the polymerization conditions and monomer formulation. Equations 4 and 5 indicate how network connectivity relates to $P_{rg}$, which can be related to the rate of erosion in photodegradable hydrogels (eq 3).

Figure 4b illustrates how $P_{rg}$ is related to the monomers or polymerization conditions for both chain- and step-polymerized hydrogels (r was assumed to be unity for all step polymerization conditions; Figure 4b). For a multifunctional monomer reacting with a difunctional monomer through step polymerization (Figure 4b, gray circles), $P_{rg}$ collapses onto the curve for the chain polymerization. However, chain polymerizations typically result in an $N$ of 10−100, while it is difficult to synthesize multifunctional monomers beyond a functionality of 8 for step polymerizations ($f_A \leq 8$). Therefore, to achieve reverse gelation points that are similar to common chain-polymerized formulations, one can copolymerize multifunctional monomers ($f_A = 3$−8) with trifunctional or tetrafunctional complementary monomers (Figure 4b; gray squares and triangles, respectively).

This analysis demonstrates how network structure relates to the rates of erosion or feature generation in photodegradable hydrogels. By exploiting the rapid erosion of step-polymerized hydrogels formed by the copolymerization of complementary tetrafunctional and difunctional monomers, photodegrading hydrogels were designed for the controlled release entrapped factors and cells29 as well as geometric patterning of cell culture microwells.34,35 Further, the increased $P_{rg}$ for CP gels is advantageous to generate materials with broad anisotropic elasticities in the $x$−$y$ plane or $z$-dimensions as the gel remains intact at a lower cross-linking density than the SP gels.

### CONCLUSION

Photodegradable hydrogels were fabricated by both chain and step polymerization from the same photolabile monomer, PEGdiPDA. Compared to chain-polymerized gels, step-polymerized hydrogels possessed increased mechanical integrity, as quantified by ductility, tensile toughness, and shear strain to yield. Increases in mechanical integrity were attributed to increased homogeneity and network cooperativity possessed in step-polymerized hydrogels as compared to the relatively heterogeneous chain-polymerized gels. Light-induced degradation and erosion was demonstrated in both the chain-polymerized and step-polymerized gels. The inherent kinetic constant of photodegradation was the same in the two systems as both gels possess the same o-nitrobenzyl ether moiety in their backbones, while the rate of erosion was much faster in step-polymerized hydrogels on account of the relatively lower network connectivity. Taken together, these studies illustrate the utility of photodegradable hydrogels polymerized by either chain or step growth polymerization and provide quantitative tools for designing unique photodegradable gels and predicting their degradation and erosion, critical parameters for regulating cell fate,37 tissue regeneration,48 and drug release among many other biomedical applications.

### AUTHOR INFORMATION

**Corresponding Author**

E-mail: (K.S.A.) kristi.anseth@colorado.edu.

**Present Address**

(A.M.K.) Departments of Chemical and Biomolecular Engineering and Materials Science and Engineering, University of Delaware, Newark, Delaware 19716, United States

**Notes**

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

The authors thank Prof. Christopher N. Bowman for generous use of the DekTak Profilometer and Dr. Kelly M. Schultz for assistance with the shear strain to yield measurements. Fellowship assistance to M.W.T. was awarded by the US Department of Education’s Graduate Assistantships in Areas of National Need, an NIH Molecular Biophysics Training Grant (T32 GM-065103), and the Teets Family Endowed Doctoral Fellowship. This work was made possible by financial support from the National Science Foundation (DMR 1006711) and the Howard Hughes Medical Institute.

### REFERENCES

1. Lutolf, M. P.; Hubbell, J. A. Nat. Biotechnol. 2005, 23, 47−55.
2. Tibbitt, M. W.; Anseth, K. S. Biotechnol. Bioeng. 2009, 103, 655−663.
3. Magin, C. M.; Finlay, J. A.; Clay, G.; Callow, M. E.; Callow, J. A.; Brennan, A. B. Biomacromolecules 2011, 12, 915−922.
4. Kidane, A.; Szabocsik, J. M.; Park, K. Biomaterials 1998, 19, 2051−2055.
5. Liechty, W. B.; Kryscio, D. R.; Slaughter, B. V.; Peppas, N. A. Annu. Rev. Chem. Biomol. Eng. 2010, 1, 149−173.
6. Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. Adv. Mater. 2006, 18, 1345−1360.
7. Sawaihny, A. S.; Pathak, C. P.; Hubbell, J. A. Macromolecules 1993, 26, 581−587.
8. Anseth, K. S.; Bowman, C. N.; Brannon-Peppas, L. Biomaterials 1996, 17, 1647−1657.
9. West, J. L.; Hubbell, J. A. Macromolecules 1999, 32, 241−244.
(10) Hoyle, C. E.; Bowman, C. N. Angew. Chem., Int. Ed. 2010, 49, 1540–1573.
(11) Anseth, K. S.; Wang, C. M.; Bowman, C. N. Polymer 1994, 35, 3243–3250.
(12) Quick, D. J.; Anseth, K. S. J. Controlled Release 2004, 96, 341–351.
(13) McCall, J. D.; Lin, C. C.; Anseth, K. S. Biomacromolecules 2011, 12, 1051–1057.
(14) Lin-Gibson, S.; Jones, R. L.; Washburn, N. R.; Horkay, F. Macromolecules 2005, 38, 2897–2902.
(15) Sakai, T.; Matsunaga, T.; Yamamoto, Y.; Ito, C.; Yoshida, R.; Suzuki, S.; Sasaki, N.; Shibayama, M.; Chung, U. I. Macromolecules 2008, 41, 5379–5384.
(16) Malkoch, M.; Vestberg, R.; Gupta, N.; Mespouille, L.; Dubois, P.; Mason, A. F.; Hedrick, J. L.; Liao, Q.; Frank, C. W.; Kingsbury, K.; Hawker, C. J. Chem. Commun. 2006, 2774–2776.
(17) Matsunaga, T.; Sakai, T.; Akagi, Y.; Chung, U.; Shibayama, M. Macromolecules 2009, 42, 1344–1351.
(18) Yang, T.; Long, H.; Malkoch, M.; Gamstedt, E. K.; Berglund, L.; Hult, A. J. Polym. Sci., Polym. Chem. 2011, 49, 4044–4054.
(19) Elbert, D. L.; Pratt, A. B.; Lustol, M. P.; Halstenberg, S.; Hubbell, J. A. J. Controlled Release 2001, 76, 11–25.
(20) Lustol, M. P.; Raebé, G. P.; Zisch, A. H.; Tirelli, N.; Hubbell, J. A. Adv. Mater. 2003, 15, 888–892.
(21) Johnson, J. A.; Baskin, J. M.; Bertozzi, C. R.; Koberstein, J. T.; Turro, N. J. Chem. Commun. 2008, 3064–3066.
(22) Johnson, J. A.; Finn, M. G.; Koberstein, J. T.; Turro, N. J. Macromolecules 2007, 40, 3589–3598.
(23) Fairbanks, B. D.; Schwartz, M. P.; Halevi, A. E.; Nuttelman, C. R.; Bowman, C. N.; Anseth, K. S. Adv. Mater. 2009, 21, S505–S510.
(24) DeForest, C. A.; Polizzotti, B. D.; Anseth, K. S. Nat. Mater. 2009, 8, 659–664.
(25) Kloxin, A. M.; Kasko, A. M.; Salinas, C. N.; Anseth, K. S. Science 2009, 324, 59–63.
(26) Frey, M. T.; Wang, Y. L. Soft Matter 2009, 5, 1918–1924.
(27) Kloxin, A. M.; Tibbitt, M. W.; Anseth, K. S. Nature Protocols 2010, 5, 1867–1887.
(28) Wong, D. Y.; Griffin, D. R.; Reed, J.; Kasko, A. M. Macromolecules 2010, 43, 2824–2831.
(29) DeForest, C. A.; Anseth, K. S. Nature Chem. 2011, 3, 925–931.
(30) Kloxin, A. M.; Benton, J. A.; Anseth, K. S. Biomaterials 2010, 31, 1–8.
(31) Kloxin, A. M.; Tibbitt, M. W.; Kasko, A. M.; Fairbairn, J. F.; Anseth, K. S. Adv. Mater. 2010, 22, 61–66.
(32) Tibbitt, M. W.; Kloxin, A. M.; Dyamenahalli, K. U.; Anseth, K. S. Soft Matter 2010, 6, 5100–5108.
(33) DeForest, C. A.; Anseth, K. S. Angew. Chem., Int. Edit. 2012, 51, 1816–1819.
(34) Peng, K.; Tomatsu, I.; van den Broek, B.; Cui, C.; Korobko, A. V.; van Noort, J.; Meijer, A. H.; Spink, H. P.; Kros, A. Soft Matter 2011, 7, 4881–4887.
(35) Tibbitt, M. W.; Han, B. W.; Kloxin, A. M.; Anseth, K. S. J. Biomed. Mater. Res. Part A 2012, 100A, 1647–1654.
(36) Fairbanks, B. D.; Singh, S. P.; Bowman, C. N.; Anseth, K. S. Macromolecules 2011, 44, 2444–2450.
(37) Bryant, S. J.; Anseth, K. S. In Scaffoldin in Tissue Engineering: Ma, P. X., Elisseef, J., Eds.; Marcel Dekker, Inc.: New York, 2005; pp 69–88.
(38) Tibbitt, M. W.; Kloxin, A. M.; Anseth, K. S. J. Polym. Sci., Polym. Chem. 2013, in press.
(39) Griffin, D. R.; Patterson, J. T.; Kasko, A. M. Biotechnol. Bioeng. 2010, 107, 1012–1019.
(40) Gould, S. T.; Darling, N. J.; Anseth, K. S. Acta Biomater. 2012, 8, 3201–3209.
(41) Flory, P. J. Principles of Polymer Chemistry, Cornell University Press: Ithaca, NY, 1953.
(42) Flory, P. J.; Rehner, J. J. Chem. Phys. 1943, 11, 512–520.
(43) Macosko, C. W.; Miller, D. R. Macromolecules 1976, 9, 199–206.