NIH Public Access
Author Manuscript
Biomaterials. Author manuscript; available in PMC 2010 April 1.

Published in final edited form as:
Biomaterials. 2009 October ; 30(30): 6048–6054. doi:10.1016/j.biomaterials.2009.07.043.

Poly(ethylene glycol) Hydrogels formed by Thiol-Ene Photopolymerization for Enzyme-Responsive Protein Delivery

Alex A. Aimetti[a], **Alexandra J. Machen**[a], and **Kristi S. Anseth**[a,b,*]

[a]Department of Chemical and Biological Engineering, University of Colorado, Boulder CO 80309, USA

[b]Howard Hughes Medical Institute, University of Colorado, Boulder CO 80309, USA

Abstract

Degradable hydrogels have been extensively used in biomedical applications such as drug delivery, and recent interest has grown in hydrogels that degrade in recognition of a cellular response. This contribution describes a poly(ethylene glycol) (PEG) hydrogel platform with human neutrophil elastase (HNE) sensitive peptide cross-links formed using thiol-ene photopolymerization rendering the gel degradable at sites of inflammation. Further, protein therapeutics can be physically entrapped within the network and selectively released upon exposure to HNE. HNE responsive hydrogels exhibited surface erosion where the degradation kinetics was influenced by changes in peptide k_{cat}, concentration of HNE, and concentration of peptide within the gel. Using this platform, we were able to achieve controlled, zero-order release of bovine serum albumin (BSA) in the presence of HNE, and release was arrested in the absence of HNE. To further exploit the advantages of surface eroding delivery systems, a smaller protein (carbonic anhydrase) was delivered at the same rate as BSA and only dependent on gel formulation and environmental conditions. Also, protein release was predicted from a 3-layered hydrogel device using mass loss data. Lastly, the bioactivity of lysozyme was maintained above 90% following the exposure to thiol-ene photopolymerization conditions.

Keywords

Hydrogel; Drug delivery; Photopolymerization; Inflammation

1. Introduction

In recent decades, hydrogels have been exploited as attractive materials for many biological applications including tissue engineering [1,2] and drug delivery [3]. Hydrogel cross-links render them insoluble, but the networks are capable of absorbing > 99% of their weight in water. The hydrophilic nature of the material is desirable for many drug delivery applications where the intended therapeutic can denature because of hydrophobic interactions [4]. In this regard, poly(ethylene glycol) (PEG) has been used extensively as a protein drug delivery platform. PEG hydrogel network properties, such as mesh size [5] and mechanical properties [6], are highly controllable. Additionally, proteins can be entrapped within this 3-dimensional

© 2009 Elsevier Ltd. All rights reserved.

*Corresponding author: Kristi.Anseth@colorado.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

network by inclusion in the prepolymer solution. Previous release studies using PEG diacrylate (PEGDA) photopolymerized hydrogels have examined the diffusion of proteins out of highly cross-linked gels [7]. The protein release is diffusion controlled, and the release rate is subsequently tuned by varying the ratio of the mesh size to the size of the protein therapeutic. However, this approach is only useful for systems when the protein size and the network mesh size are similar. More recently, efforts have expanded in the synthesis of responsive hydrogels that are capable of releasing a protein upon an applied stimulus such as pH [8], temperature [9], glucose [10], or enzymatic activity [11]. While these materials provide a biocompatible platform with the potential for controlled release applications, there remains a need for biomaterials to respond to cellular stimuli.

As an alternative to diffusion-controlled release, researchers are interesting in designing intelligent drug delivery systems capable of achieving localized, controlled release of a therapeutic in recognition of a cellular event. One current approach involves covalently incorporating enzyme cleavage peptides as pendent groups within the network [12-14]. While these systems allow elegant control of the release for small biomolecules, the amount of drug that can be loaded within the hydrogel is limited. Also, the gel must allow for adequate enzyme diffusion, which can affect which protease is targeted or dictate the gel structural properties needed to allow for rapid diffusion. Alternatively, peptides have been incorporated as cross-links within PEG hydrogels rendering the material degradable upon exposure to a particular enzyme. Collagenase sensitive peptides have been incorporated within a PEGDA monomer prior to gel formation to promote encapsulated cell migration [15]. The advantage of this cell-responsive material is the method by which it is fabricated. Photopolymerization serves as an efficient method for gel preparation. The photoinitiated reaction occurs under mild, aqueous conditions at room temperature with spatial and temporal control over the reaction kinetics. These reaction conditions allow for the preservation of therapeutic efficacy during gel formation. Also, photopolymerization allows for facile *in situ* gel formation and the ability to fabricate complex geometric devices. However, some of the limitations of this material platform are the requirement for post-synthetic modification of the peptide for monomer preparation and the heterogeneous network structure that results from acrylate photopolymerization [16].

Another enzyme-responsive, PEG-based hydrogel platform that has been used for protein release relies on a Michael-type reaction for gel formation [11,17,18]. A multi-arm PEG containing terminal vinyl groups can react selectively with a bis-cysteine peptide under basic conditions. This reaction serves as an effective way to incorporate enzyme cleavable peptides within hydrogel backbones by exploiting the sulfhydryl group on cysteine residues. In addition, this reaction proceeds in a step-wise growth mechanism resulting in an ideal, homogeneous network. However, there is minimal control over the reaction in space and time. In an attempt to utilize the advantages of both hydrogel platforms with peptide cross-links, thiol-ene photopolymerization has been explored as a novel way to fabricate PEG-based hydrogels [19].

Thiol-ene photopolymerization is a highly efficient, radical mediated reaction between a thiol and an alkene. A radical source, or photoinitiator in the presence of light, abstracts a hydrogen from a thiol forming a thiyl radical which can add across a carbon-carbon double bond. The subsequent propagation-chain transfer events result in a step-growth mechanism [20]. Using this reaction mechanism for hydrogel formation allows for spatial and temporal control of polymerization, a resulting homogeneous network structure, and the ability to easily incorporate enzymatically degradable peptides within the backbone rendering the gel responsive to cellular events.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

This contribution presents the use of thiol-ene photopolymerization as a method for fabricating enzyme-responsive PEG hydrogels for the application of controlled protein release. In specific, we are interested in developing a material with the ability to treat inflammation locally. Previous research has examined incorporating human neutrophil elastase (HNE), an enzyme present during inflammation, substrates within PEG hydrogels for controlled enzyme dictated release [12]. This work examines the effect of HNE substrate (k_{cat}), concentration of HNE, and concentration of substrate within the gel on the kinetics of enzyme triggered gel degradation and subsequent protein release. The controlled, predictable delivery of a model protein (bovine serum albumin – BSA) was tailored by variations in hydrogel formulation. Lastly, the effect of thiol-ene photopolymeriation on the bioactivity of an encapsulated protein was examined.

2. Experimental Section

Materials

4-arm poly(ethylene glycol) (PEG) was obtained from JenKem Technology USA. 5-Norbornene-2-carboxylic acid was purchased from Sigma-Aldrich. Fmoc protected amino acids in their L-configuration, as well as *O*-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) used for amino acid activation, were obtained from Anaspec. MBHA Rink Amide resin was purchased from Novabiochem. Bovine serium albumin (BSA), carbonic anhydrase (CA), and rhodamine B isothiocyanate (RBITC) were obtained from Sigma-Aldrich. Lysozyme and *micrococcus lysodeikticus* were purchased from Sigma-Aldrich and Worthington Biochemical Corp., respectively. Human neutrophil elastase (HNE) was supplied as a lyophilized powder from Innovative Research.

4-arm PEG-norbornene (PEG5k-norbornene) synthesis

4-arm PEG (M_n~5,000) with terminal hydroxyls (-OH) was functionalized with norbornenes as follows. 5-norbornene-2-carboxcylic acid (24 mmol, 2.94 mL) and N,N'-diisopropylcarbodiimide (DIC) (12 mmol, 1.86 mL) were reacted in dichloromethane (DCM) for 30 minutes to form the anhydride. In a separate round bottom flask (dried overnight), 4-arm PEG-OH (4 mmol, 5 g) was dissolved in 40 mL DCM. To that, pyridine (20 mmol, 1.62 mL) and 4-dimethylaminopyridine (DMAP) (2 mmol, 0.24 g) were added. The norbornene anhydride was then transferred to the PEG solution using a canula. The reaction was allowed to proceed overnight at room temperature under an inert atmosphere. Afterwards, the reaction was filtered, concentrated under rotary evaporation, and precipitated and washed (3x) in chilled diethyl ether. The product was verified with H1 NMR (Supporting Information, Fig. S1). ^{1}H NMR (500 MHz, CDCl3) δ 6.28-5.79 (m, 2H), 4.24 – 4.09 (m, 2H), 3.78 – 3.43 (m, 112H).

Peptide synthesis

HNE sensitive peptide sequences were synthesized (Protein Technologies, Inc Tribute Peptide Synthesizer) using solid phase Fmoc chemistry on a MHBA Rink Amide Resin (~0.7 mmol/g resin substitution). Amino acid coupling was performed using HBTU/N-methylmorpholine (NMM) in N-methylpyrolidone (NMP). Peptides were cleaved from the resin using trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/water/D,L-dithiothreitol (DTT) (95/2.5/2.5 v/v) and allowed to react at room temperature for 2h. The reaction was filtered and the filtrate precipitated and washed (3x) in chilled diethyl ether. Peptides were purified by semi-preparative reversed phase HPLC (Waters Delta Prep 4000) using a 70-min linear (5-95%) gradient of acetonitrile in 0.1% trifluoroacetic acid. Peptide purity was confirmed by analytical reversed phase HPLC C18 column and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Applied Biosystem DE Voyager)(Supporting Information, Fig. S2).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Thiol-ene photopolymerization of PEG-based hydrogels

PEG-peptide hydrogels were formed via thiol-ene photopolymerization (Fig. 1). Due to the step-wise mechanism of network development (REF Ben), thiol and norbornene functionalities were combined in equimolar concentrations. PEG5k-norbornene and bis-cysteine HNE sensitive peptide accounted for 10% (w/w) (roughly 60 mM of functional groups) of the monomer solution. To that, the photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (I-2959, Ciba-Geigy) was added at a final concentration of 0.1 wt%. The viscous solution was exposed to 365 nm light at an intensity of ~10 mW cm^{-2} for 10 minutes. Gelation time and final elastic modulus were determined by dynamic time sweep rheology experiments using a modified ARES rotational rheometer (TA Instruments) operating in the linear viscoelastic region (strain=1%, frequency=100 rad s^{-1}).

Hydrogel mass loss studies

Hydrogel disks (diameter=5.1 mm, thickness=0.9 mm) were placed in buffer (0.05 M HEPES pH 7.4 + 150 mM NaCl) at 37°C. HNE was added to initiate hydrogel degradation. For experiments examining the kinetic effect of the HNE sensitive peptide and PEG molecular weight on gel degradation, the final concentration of HNE added was 1μM. For experiments exceeding 2 h, the samples remaining were placed in fresh buffer and HNE to account for the half-life of the enzyme *in vitro* ($t_{1/2}$~30min as determined using a commercially available HNE substrate). During gel degradation, samples were removed at their respective time points, washed (3x) in H_2O, freeze dried for 24 h, and then weighed to determine the mass loss to that point.

Protein release experiments

Rhodamine-labeled protein BSA was used as a model protein to characterize release from the PEG-based hydrogels formed via thiol-ene photopolymerization. Prior to gel formation, BSA was added to the monomer solution described earlier at a final concentration of 1 mg mL^{-1}. After photopolymerization, gels were placed in buffer at 37°C for 2 h to release any protein present on the surface of the gel. HNE was added to initiate protein release from the network. At predetermined time points, the release medium was sampled and replenished with fresh buffer and HNE. Fluorescence was measured (λ_{ex}=550 nm λ_{em}=590 nm, Perkin Elmer Wallac Victor^2 1420 Multilabel Counter) to quantitate protein release.

Protein release from layered hydrogel

A three-layered hydrogel (diameter=5.1mm, thickness=1.2 mm, each layer thickness=0.4 cm) was formed via sequential photopolymerizations. The bottom and top layers were fabricated with a gel formulation comprised of PEG5k-norbornene and CGAAPVRGGGGC while the middle layer contained the monomers, PEG5k-norbornene and CGAAP(Nva)GGGGGC. Each prepolymer solution contained BSA-rhodamine (1mg mL^{-1}). 1μM HNE was added and fluorescence detected to determine protein release.

Bioactivity of encapsulated protein

Lysozyme was used as a model protein to determine if the radical-based, thiol-ene reaction had deleterious effects on the activity of the encapsulated biomolecule. Monofunctionalized PEG5k-norbornene (MeO-PEG5k-norbornene) and bis-cysteine peptide were used to achieve similar concentrations of active radicals during photopolymerization without achieving a 3-dimensional, crosslinked network. The prepolymer solution consisted of 10 wt% monomer (MeO-PEG5k-norbornene & peptide) and 2 mg mL^{-1} lysozyme. The solution was exposed to 365 nm light (~10 mW cm^{-2}) for 10 minutes to mimic hydrogel formation conditions. The solution was diluted 100-fold to assay for lysozyme bioactivity. Relative bioactivity was normalized to the positive control (native lysozyme solution).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

3. Results and Discussion

3.1 Rheological study of hydrogel polymerization via thiol-ene photochemistry

Fig. 2 shows the time sweep measurement of the storage modulus (G') and loss modulus (G") over the time course of gel formation when irradiated with UV light. Crossover point for gelation (an estimate of gel point conversion) was determined at the point when G'>G" [21]. Based on this information, gelation occurs within 1 minute of light exposure and the gels reach a shear elastic modulus of ~6 kPa under typical photoinitation conditions (0.1 wt% I-2959, 365 nm light, 10 min). Gel mechanical properties can be furthered tailored by varying the functionality or molecular weight of the starting macromolecules. For example, the shear elastic modulus decreased to ~1 kPa when a 4-arm PEG-norbornene (M_n~20,000, PEG_{20k}-nobornene) was used in the gel formulation (Supporting Information, Fig. S3). Gelation time and properties were unaffected with the incorporation of protein in the prepolymer solution (Supporting Information, Fig. S4).

3.2 Characterization of HNE triggered gel degradation

Hubbell and coworkers demonstrated that enzyme-responsive hydrogel degradation is dependent on the peptide reaction constant (k_{cat}), concentration of enzyme ([E]), and concentration of substrate within the intact gel ($[S]_o$) [11]. Fig. 3 shows the kinetics of hydrogel mass loss in the presence of HNE. Varying the HNE-sensitive peptide cross-link affects the kinetics of enzyme reaction necessary to degrade the gel. Gels with the HNE substrate CGAAPVRGGGC degraded within 2 hours of enzyme exposure. However, when CGAAP(Nva)GGGGC was used as a peptide cross-link a 2.5 fold increase in degradation time was observed likely due to the decrease in k_{cat} [12]. Decreasing the concentration of HNE can further reduce the kinetics of enzyme reaction. Fig. 3b illustrates a 3-fold delay in gel degradation in the presence of 200 nM HNE compared to 1 μM HNE. Since these gels are made with stoichiometric equivalents of norbornene to thiols, an increase in molecular weight of PEG monomer inherently decreases the concentration of substrate within the hydrogel. Fig. 3c shows the accelerated gel degradation when the peptide crosslink density is decreased.

It is also important to note that the mechanism for gel degradation is surface erosion as indicated by the linearity of the mass loss profiles. To further confirm the mode of degradation, the equilibrium swelling ratio was monitored over time in the presence of enzyme. Fig. 3d is a representative graph showing constant swelling ratio over the course of gel degradation.

3.3 HNE triggered release of encapsulated protein from PEG hydrogels

To demonstrate the utility of this hydrogel platform for drug delivery applications, BSA was encapsulated and subsequently released upon gel degradation. Fig. 4a shows varying protein release curves when the HNE substrate was altered. No BSA release was observed over the time scale of the experiment in the absence of HNE (data not shown). To further show the triggerable release mechanism, Fig. 4b illustrates the on-off temporal control of protein delivery only in the presence of HNE.

Surface eroding delivery platforms are capable of releasing molecules, of varying sizes, at the same rate dictated by the gel formulation and the environmental conditions [22]. Fig. 5 illustrates the similar release profile of a smaller protein, carbonic anhyrdrase (CA ~ 29 kDa) to that of BSA (~66 kDa) and the correlation with gel mass loss under the same experimental parameters.

The protein release profile is dictated solely by gel degradation kinetics, which is characteristic of surface eroding delivery systems.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

3.4 Predicting protein release from tri-layered hydrogel formed with thiol-ene photopolymerization

Extensive work has been done to develop models able to predict drug release from surface eroding systems [23,24]. As shown previously, mass loss data agrees with fractional release profiles. Therefore, by predicting mass loss we can accurately describe the release of a protein from a surface eroding system. The kinetics of mass loss is proportional to the surface area of the device. In the case of thin hydrogel disks, the surface area remains relatively unchanged throughout degradation (i.e., 1D erosion). Mass loss can then be predicted with the following equations,

$$\frac{df}{dt}=k,$$

where f is the fractional mass loss and k (hr^{-1}) is the slope. Assuming constant density and surface area, a change in the sample dimension in the direction of degradation front (i.e., thickness) is decreased as,

$$-\frac{dl}{dt}=k',$$

where l is the length in a geometric direction and k' is the degradation constant (mm hr^{-1}) and,

$$k'=kl_o,$$

where l_o is the original hydrogel thickness (mm).

Fig. 6 illustrates the ability to predict protein release from surface eroding, enzyme-responsive hydrogels formed via thiol-ene photopolymerization. The outer layers comprise of roughly 66% of the total volume of the gel. Up to that point, the mass loss is predicted using a single rate of degradation (k'=0.41 mm hr^{-1})(Fig. 6, solid line). Upon reaching the inner layer containing a different peptide cross-link, a unique degradation rate constant (k'=0.18 mm hr^{-1}) was implemented to achieve a decreased rate of release (Fig. 6, dashed line). By solely changing the geometry with materials of varying degradation kinetic constants, we have obtained a bimodal release profile with highly predictable kinetics.

3.5 Effect of thiol-ene photopolymerization on the bioactivity of lysozyme

Importantly, lysozyme activity was maintained above ~93% when exposed to the same conditions as thiol-ene photopolymerization for gel formation in the presence of macromers (Fig. 7). Free radicals formed by bond photolysis of the initiator when exposed to UV light attacked the protein in the absence of macromers significantly reducing the bioactivity to roughly 12% and 4% in the presence of 0.1 wt% and 0.025 wt% I-2959, respectively, after 10 min.

Discussion

The scope of this contribution was to describe and characterize a method for hydrogel formation using thiol-ene photopolymerization for applications in controlled, enzyme-responsive protein delivery. HNE was chosen as a targeted enzyme due to its high specificity to sites of inflammation. During the onset of inflammation, neutrophils respond to infection by releasing a variety of biomolecules, including enzymes such as HNE. Here we present a hydrogel drug

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

delivery platform with HNE-sensitive peptide crosslinks, which allow for gel degradation and subsequent protein release upon exposure to HNE. These HNE-responsive gels exhibited surface erosion in the presence of enzyme. Classic surface eroding platforms, such as polyanhyrides [25,26], exhibit surface erosion because the diffusion of water within the hydrophobic polymer is much slower than the kinetics of bond hydrolysis at the material surface. Similarly, the diffusion of HNE (~29 kDa protein) within the matrix is severely hindered, if not completely inhibited in these cross-linked gels. The mesh size of the thiol-ene gels was approximated using rubber elasticity theory [27-29] and found to be on the same order of magnitude as the hydrodynamic radius of HNE. The timescale for reaction appears to be faster than the timescale for molecular diffusion within the cross-linked hydrogel resulting in surface erosion.

Surface eroding biomaterials can be highly desirable for drug delivery application. As shown previously, protein release rates are not dependent on the size of the protein. In diffusion controlled drug delivery systems, the radius of the protein plays a critical role in the rate at which it is released. However, in an enzymatically reaction controlled delivery system, the release of various biomolecules is dependent on factors influencing degradation (k_{cat}, [E], $[S]_o$). By solely changing the gel formulation, highly controlled, tailorable zero-order release can be obtained. This characteristic can be exploited in a multi-layered hydrogel platform formed by subsequent photopolymerizations. This layered device could also be used to achieve sequential, controlled delivery of multiple proteins where the release can be predicted based on 1-D mass loss experiments.

Although photopolymerization reaction conditions are considered mild, it is still important to consider the potential high-energy free radical interaction with encapsulated protein therapeutics. Lin et al. hypothesized mechanisms of protein inactivation exposed to acrylate photopolymerization [30]. In addition, (meth)acrylates non-specifically react with proteins in the presence of a radical source, which could affect the bioactivity [31,32]. The potential advantage of thiol-ene photopolymerization for protein encapsulation is the minimal interaction the monomers would have with the protein. Most of the cysteine residues are disulfide bridged within proteins resulting in minimal free thiols. Additionally, if modification of the protein did occur through an available thiol, PEGylation of a protein has been shown to enhance its stability in circulation [33,34]. BSA is known to contain 1 free thiol; however, the crystal structure shows that it is not located at the surface indicating it would not participate in the photoreaction of macromers [35]. Photorheology of a prepolymer solution containing BSA was examined (Supporting Information Fig. S2). The final elastic modulus was similar for thiol-ene gels formed in the presence or absence of BSA suggesting minimal interactions with the protein during photopolymerization. The advantages of forming hydrogels for drug delivery applications via photopolymerization are well known, however, it is still important to select a reaction mechanism capable of rapidly forming a hydrogel with minimal deleterious interactions with the protein.

Conclusion

Thiol-ene photopolymerization for biomedical applications was recently presented as a novel mechanism for hydrogel formation. We exploited this technique to fabricate protein delivery vehicles capable of enzyme responsive protein release at sites of inflammation. Controlled, tailorable mass loss profiles exhibiting surface erosion were obtained by varying k_{cat}, concentration of HNE, and concentration of substrate. The advantages of surface eroding platforms were exploited to release various size proteins on the same time scale dictated by gel formulation. Additionally, the release can easily be predicted from more complex hydrogels using 1-dimensional mass loss data. And finally, lysozyme retains ~93% of its bioactivity post thiol-ene photopolymerization. This delivery platform has potential application for treatment

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

of disease where inflammation has been implicated such as diabetes [36], rheumatoid arthritis [37], and cancer [38].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Ben Fairbanks and Cole DeForest for helpful discussions involving the thiol-ene reaction mechanism. Also, we would like to thank Dr. Chien-Chi Lin for discussions involving the bioactivity of encapsulated proteins. Additionally, the authors thank NIH (Grant RO1 DK076084), HHMI, Graduate Assistance in Areas of National Need (A.A.A.), and the NSF-REU program (A.J.M.) for financial support of this research.

References and Notes

1. Burdick JA, Anseth KS. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. Biomaterials 2002;23(22):4315–23. [PubMed: 12219821]
2. Nguyen KT, West JL. Photopolymerizable hydrogels for tissue engineering applications. Biomaterials 2002;23(22):4307–14. [PubMed: 12219820]
3. Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. European Journal of Pharmaceutics and Biopharmaceutics 2000;50(1):27–46. [PubMed: 10840191]
4. Lin CC, Metters AT. Hydrogels in controlled release formulations: Network design and mathematical modeling. Advanced Drug Delivery Reviews 2006;58(1213):1379–408. [PubMed: 17081649]
5. Lu SX, Anseth KS. Release behavior of high molecular weight solutes from poly(ethylene glycol)-based degradable networks. Macromolecules 2000;33(7):2509–15.
6. Bryant SJ, Bender RJ, Durand KL, Anseth KS. Encapsulating Chondrocytes in degrading PEG hydrogels with high modulus: Engineering gel structural changes to facilitate cartilaginous tissue production. Biotechnology and Bioengineering 2004;86(7):747–55. [PubMed: 15162450]
7. Mellott MB, Searcy K, Pishko MV. Release of protein from highly cross-linked hydrogels of poly (ethylene glycol) diacrylate fabricated by UV polymerization. Biomaterials 2001;22(9):929–41. [PubMed: 11311012]
8. Sawhney AS, Pathak CP, Hubbell JA. Bioerodible Hydrogels Based on Photopolymerized Poly (Ethylene Glycol)-Co-Poly(Alpha-Hydroxy Acid) Diacrylate Macromers. Macromolecules 1993;26 (4):581–87.
9. Bromberg LE, Ron ES. Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. Advanced Drug Delivery Reviews 1998;31(3):197–221. [PubMed: 10837626]
10. Qiu Y, Park K. Environment-sensitive hydrogels for drug delivery. Advanced Drug Delivery Reviews 2001;53(3):321–39. [PubMed: 11744175]
11. Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, et al. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics. Proceedings of the National Academy of Sciences of the United States of America 2003;100(9):5413–18. [PubMed: 12686696]
12. Aimetti AA, Tibbitt MW, Anseth KS. Human neutrophil elastase responsive delivery from poly (ethylene glycol) hydrogels. Biomacromolecules 2009;10(6):1484–9. [PubMed: 19408953]
13. Zisch AH, Schenk U, Schense JC, Sakiyama-Elbert SE, Hubbell JA. Covalently conjugated VEGF-fibrin matrices for endothelialization. Journal of Controlled Release 2001;72(13):101–13. [PubMed: 11389989]
14. Sakiyama-Elbert SE, Panitch A, Hubbell JA. Development of growth factor fusion proteins for cell-triggered drug delivery. Faseb Journal 2001;15(7):1300–02. [PubMed: 11344120]
15. Mann BK, Gobin AS, Tsai AT, Schmedlen RH, West JL. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ECM analogs for tissue engineering. Biomaterials 2001;22(22):3045–51. [PubMed: 11575479]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

16. Lin-Gibson S, Jones RL, Washburn NR, Horkay F. Structure-property relationships of photopolymerizable poly(ethylene glycol) dimethacrylate hydrogels. Macromolecules 2005;38(7): 2897–902.

17. Elbert DL, Pratt AB, Lutolf MP, Halstenberg S, Hubbell JA. Protein delivery from materials formed by self-selective conjugate addition reactions. Journal of Controlled Release 2001;76(12):11–25. [PubMed: 11532309]

18. Lutolf MP, Raeber GP, Zisch AH, Tirelli N, Hubbell JA. Cell-responsive synthetic hydrogels. Advanced Materials 2003;15(11):888–+.

19. Fairbanks MP BDS, Halevi AE, Nuttelman CR, Bowman CN, Anseth KS. A versatile synthetic extracellular matrix mimic via thiol-ene photopolymerization. In Review

20. Cramer NB, Bowman CN. Kinetics of thiol-ene and thiol-acrylate photopolymerizations with real-time Fourier transform infrared. Journal of Polymer Science Part a-Polymer Chemistry 2001;39(19): 3311–19.

21. Tung CYM, Dynes PJ. Relationship between Viscoelastic Properties and Gelation in Thermosetting Systems. Journal of Applied Polymer Science 1982;27(2):569–74.

22. Quick DJ, Macdonald KK, Anseth KS. Delivering DNA from photocrosslinked, surface eroding polyanhydrides. Journal of Controlled Release 2004;97(2):333–43. [PubMed: 15196760]

23. Hopfenberg HB. Controlled Release from Erodible Slabs, Cylinders, and Spheres. Acs Symposium Series 1976;33:26–32.

24. Gopferich A, Karydas D, Langer R. Predicting Drug-Release from Cylindric Polyanhydride Matrix Discs. European Journal of Pharmaceutics and Biopharmaceutics 1995;41(2):81–87.

25. Muggli DS, Burkoth AK, Anseth KS. Crosslinked polyanhydrides for use in orthopedic applications: Degradation behavior and mechanics. Journal of Biomedical Materials Research 1999;46(2):271–78. [PubMed: 10380006]

26. Burkoth AK, Anseth KS. A review of photocrosslinked polyanhydrides: in situ forming degradable networks. Biomaterials 2000;21(23):2395–404. [PubMed: 11055287]

27. Canal T, Peppas NA. Correlation between Mesh Size and Equilibrium Degree of Swelling of Polymeric Networks. Journal of Biomedical Materials Research 1989;23(10):1183–93. [PubMed: 2808463]

28. Lustig SR, Peppas NA. Solute Diffusion in Swollen Membranes .9. Scaling Laws for Solute Diffusion in Gels. Journal of Applied Polymer Science 1988;36(4):735–47.

29. Anseth KS, Bowman CN, BrannonPeppas L. Mechanical properties of hydrogels and their experimental determination. Biomaterials 1996;17(17):1647–57. [PubMed: 8866026]

30. Lin CC, Sawicki SM, Metters AT. Free-radical-mediated protein inactivation and recovery during protein photoencapsulation. Biomacromolecules 2008;9(1):75–83. [PubMed: 18088094]

31. Lin CC, Metters AT. Enhanced protein delivery from photopolymerized hydrogels using a pseudospecific metal chelating ligand. Pharm Res 2006;23(3):614–22. [PubMed: 16397740]

32. Bailon P, Palleroni A, Schaffer CA, Spence CL, Fung WJ, Porter JE, et al. Rational design of a potent, long-lasting form of interferon: a 40 kDa branched polyethylene glycol-conjugated interferon alpha-2a for the treatment of hepatitis C. Bioconjug Chem 2001;12(2):195–202. [PubMed: 11312680]

33. Diwan M, Park TG. Pegylation enhances protein stability during encapsulation in PLGA microspheres. Journal of Controlled Release 2001;73(23):233–44. [PubMed: 11516501]

34. Hinds KD, Kim SW. Effects of PEG conjugation on insulin properties. Advanced Drug Delivery Reviews 2002;54(4):505–30. [PubMed: 12052712]

35. Hayashi T, Shimoyama Y, Kuwata K, Era S. Investigation of slow dynamics of the sulfhydryl in the solution and gel states of bovine serum albumin: A vector electron paramagnetic resonance study. Japanese Journal of Physiology 1999;49(1):27–33. [PubMed: 10219106]

36. Koulmanda M, Bhasin M, Hoffman L, Fan ZG, Qipo AD, Shi H, et al. Curative and beta cell regenerative effects of alpha 1-antitrypsin treatment in autoimmune diabetic NOD mice. Proceedings of the National Academy of Sciences of the United States of America 2008;105(42):16242–47. [PubMed: 18852471]

37. Choy EHS, Panayi GS. Mechanisms of disease: Cytokine pathways and joint inflammation in rheumatoid arthritis. New England Journal of Medicine 2001;344(12):907–16. [PubMed: 11259725]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

38. Schafer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nature Reviews Molecular Cell Biology 2008;9(8):628–38.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

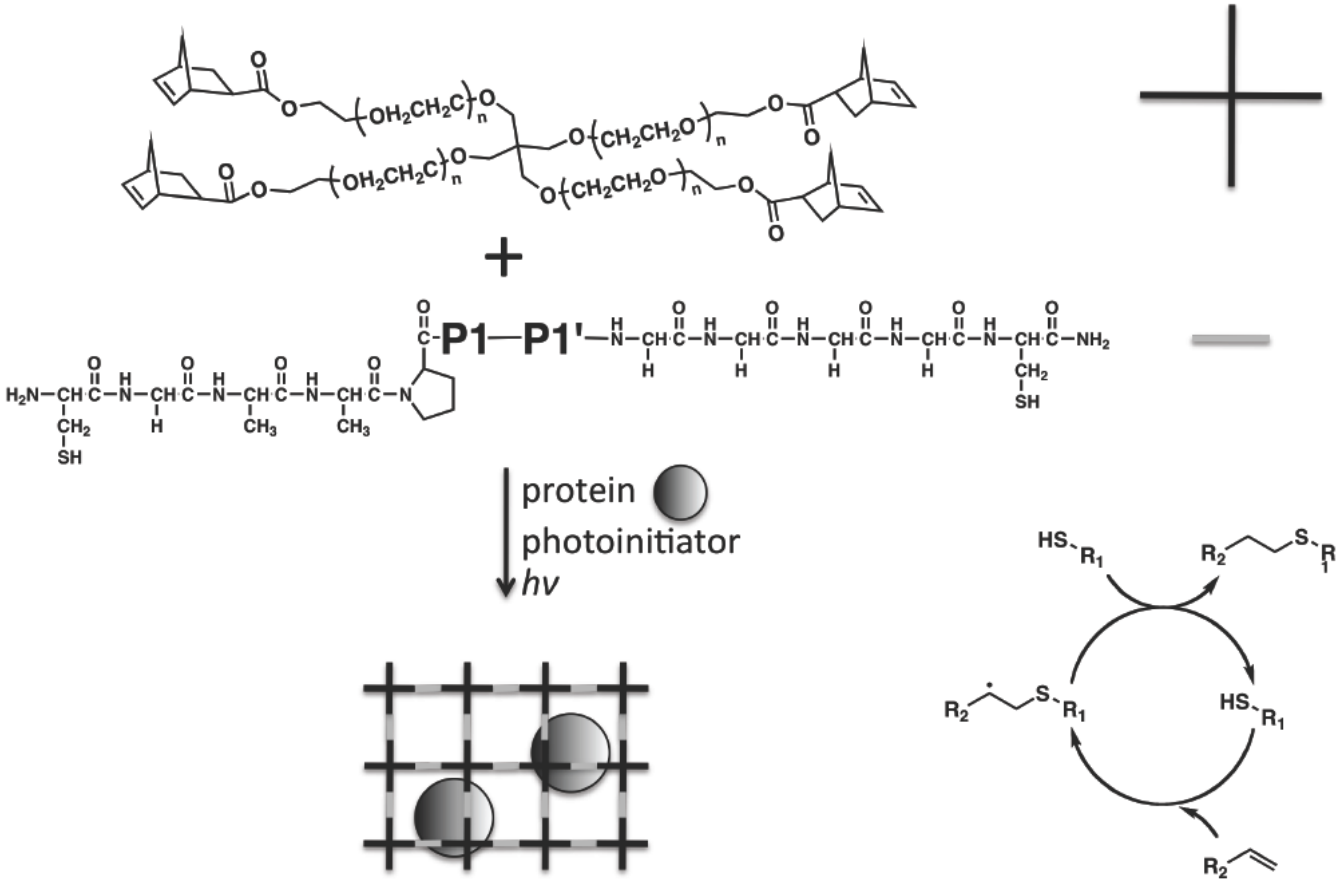

Fig. 1.

Schematic illustrating the design and formation of enzyme-responsive PEG hydrogels fabricated via thiol-ene photopolymerization (reaction mechanism included) for the controlled release of protein therapeutics. Enzyme cleavage occurs between the P1 and P1' amino acid residues.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

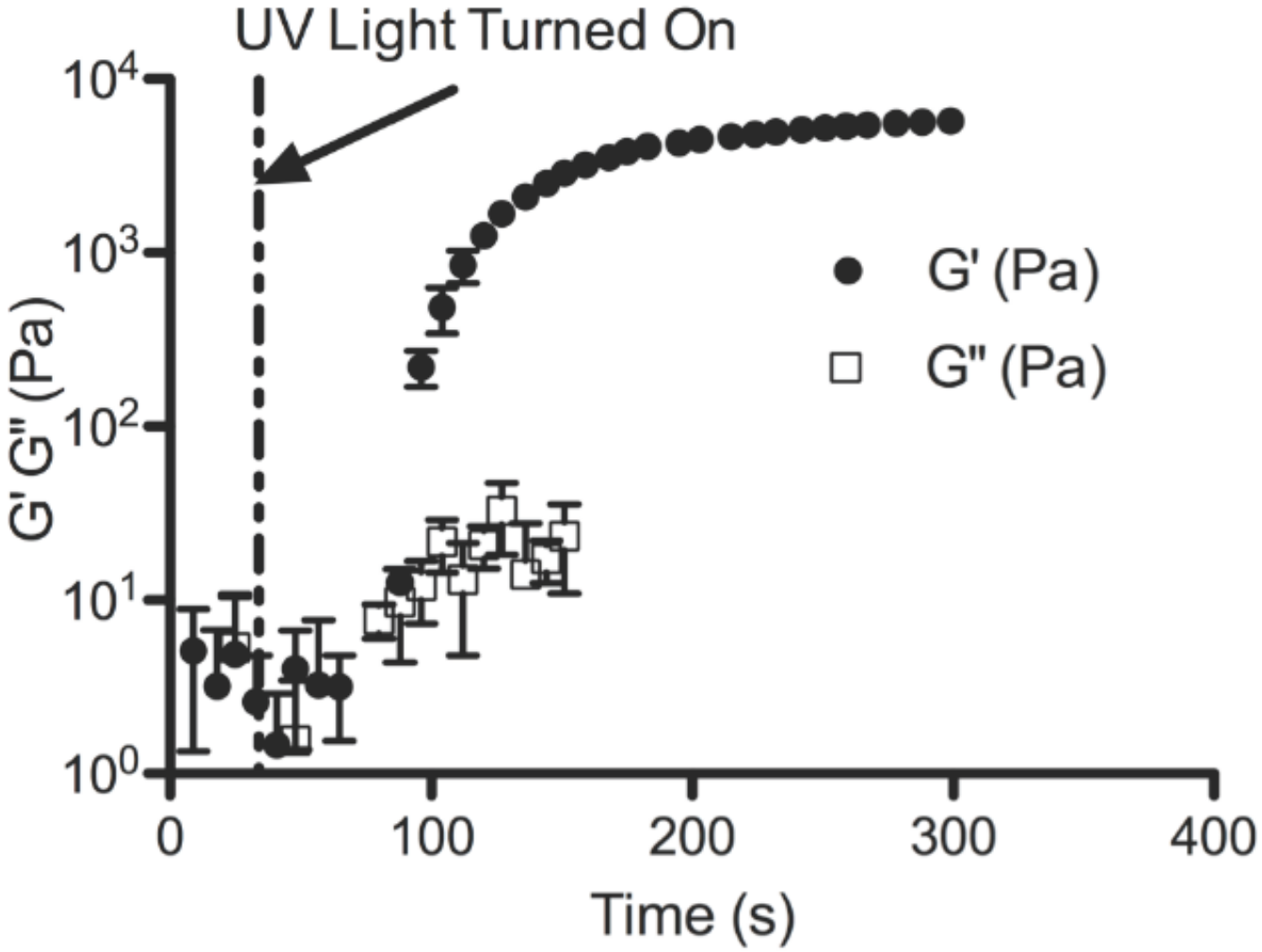

Fig. 2.

Dynamic time sweep photorheology of thiol-ene photopolymerization hydrogel formation with PEG_{5k}. Gel point is approximated when the storage modulus (G', ●) overcomes the loss modulus (G", □). Dashed line (---) illustrates when the UV light was turned on to initiate polymerization.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

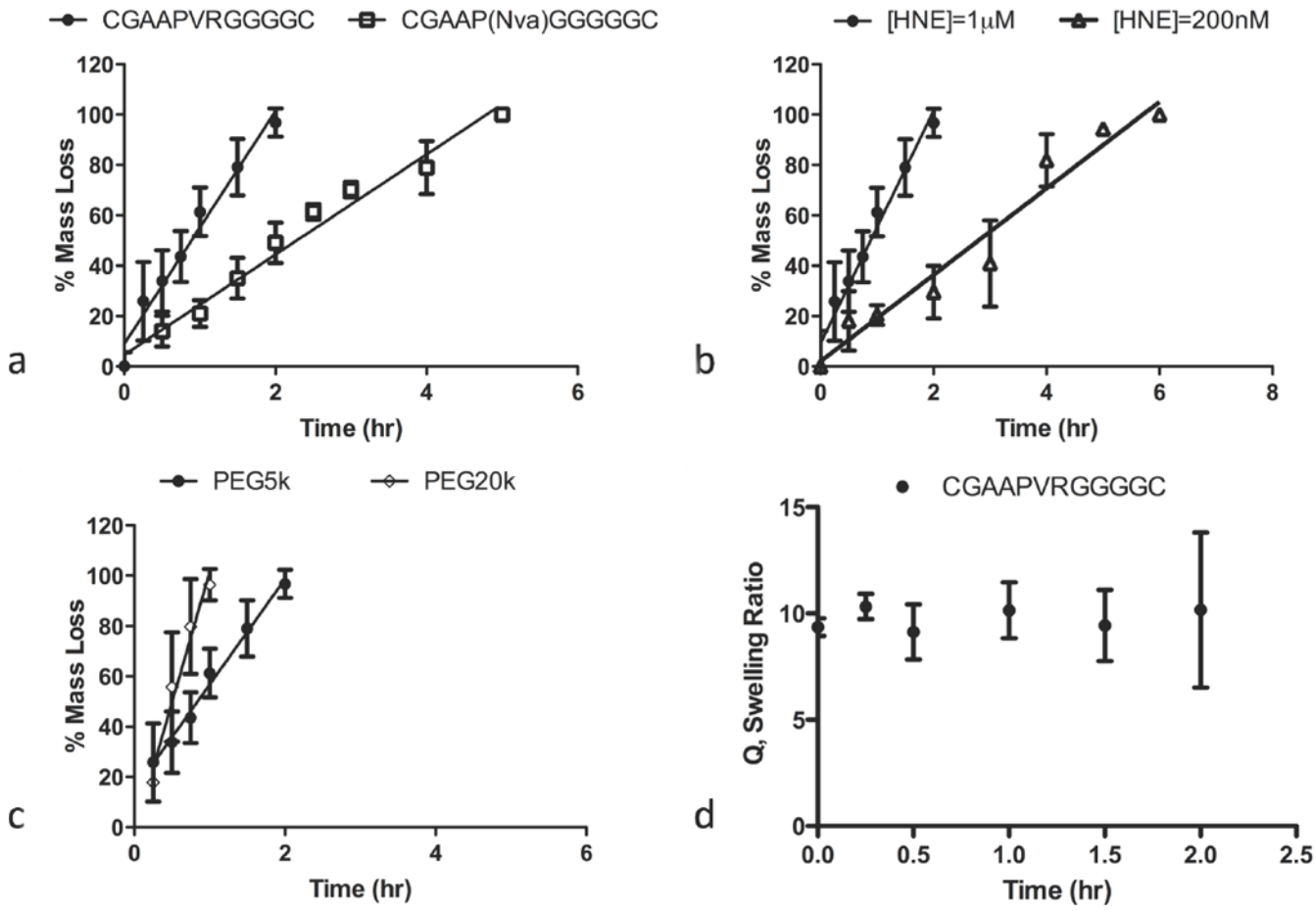

Fig. 3.

Gel mass loss profiles upon exposure to HNE (A-C). Influence of HNE peptide cross-link (A), concentration of HNE (B), and concentration of substrate (C) were experimentally studied. (D) Representative equilibrium swelling ratio as a function of time in the presence of HNE. All gels were made of 10 wt% monomer and exposed to 1μM HNE (unless otherwise noted).

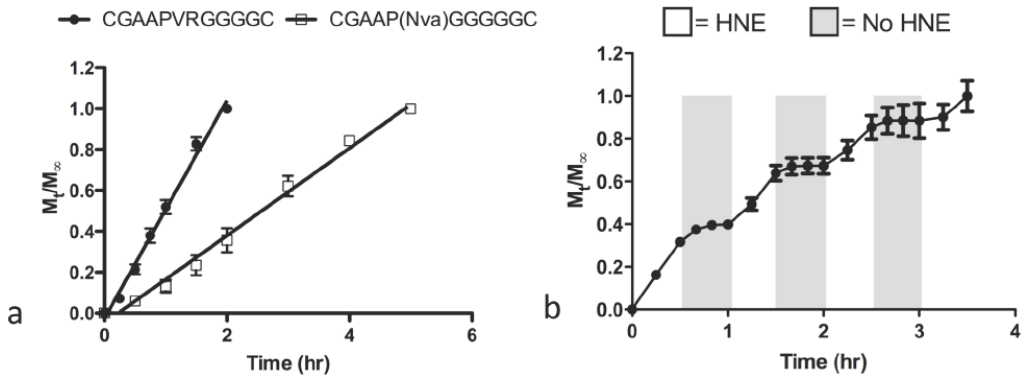

Fig. 4.

BSA release profiles in the presence of 1μM HNE. (A) Protein release from thiol-ene hydrogels fabricated with varying peptide cross-links. (B) Temporal control of protein release when HNE is dosed (□) and when it is removed (■). HNE substrate: CGAAPV↓RGGGGC.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

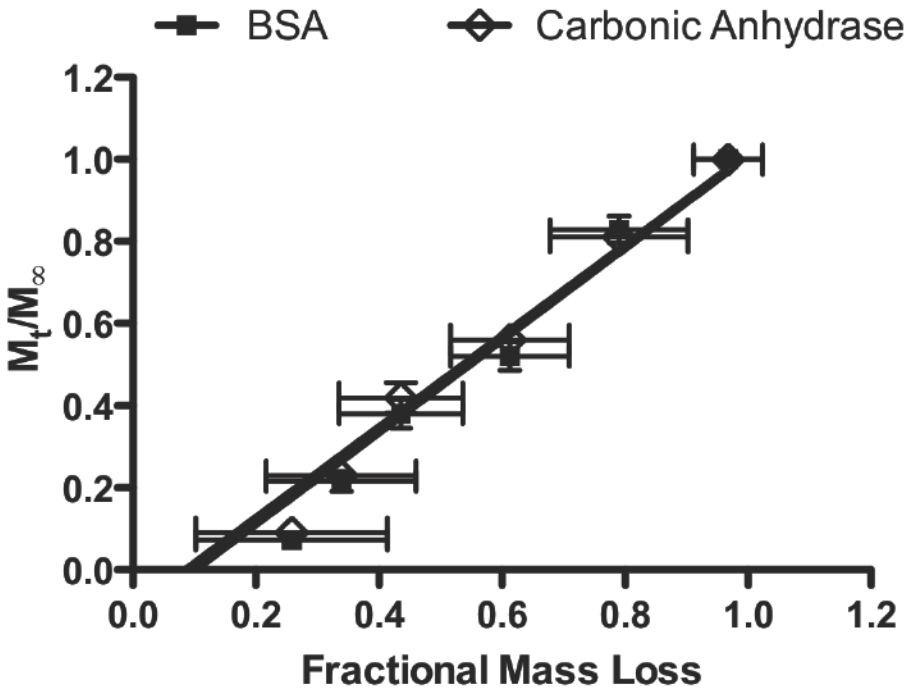

Fig. 5.

Protein release from thiol-ene hydrogels as a function of fractional mass loss. Each data point represents the same time. Gel formulation: PEG_{5k}-norbornene + CGAAPVRGGGC + protein (1mg mL^{-1}). [HNE] = 1 μM.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

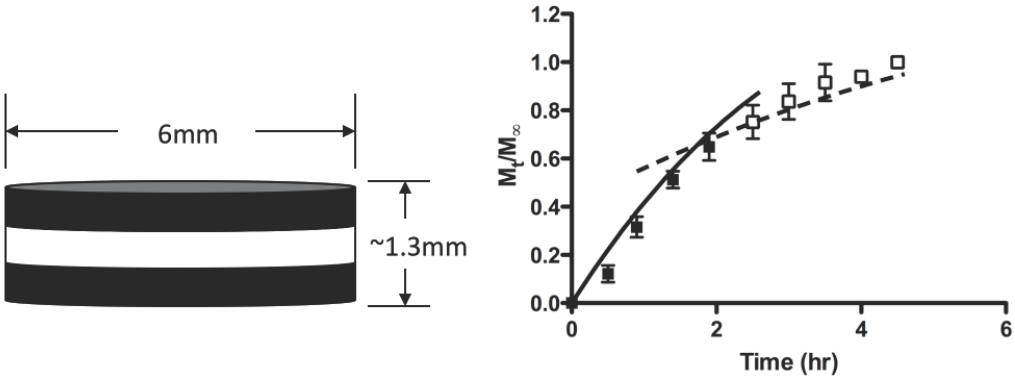

Fig. 6.

BSA release from 3-layered hydrogel formed by subsequent thiol-ene photopolymerizations. ■ = PEG_{5k}-norbornene + CGAAPVRGGGC + BSA (1 mg mL^{-1}), □ = PEG_{5k}-norbornene + CGAAP(Nva)GGGGC + BSA (1 mg mL^{-1}). Solid (—) and dashed (- - -) lines represent predicted release.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

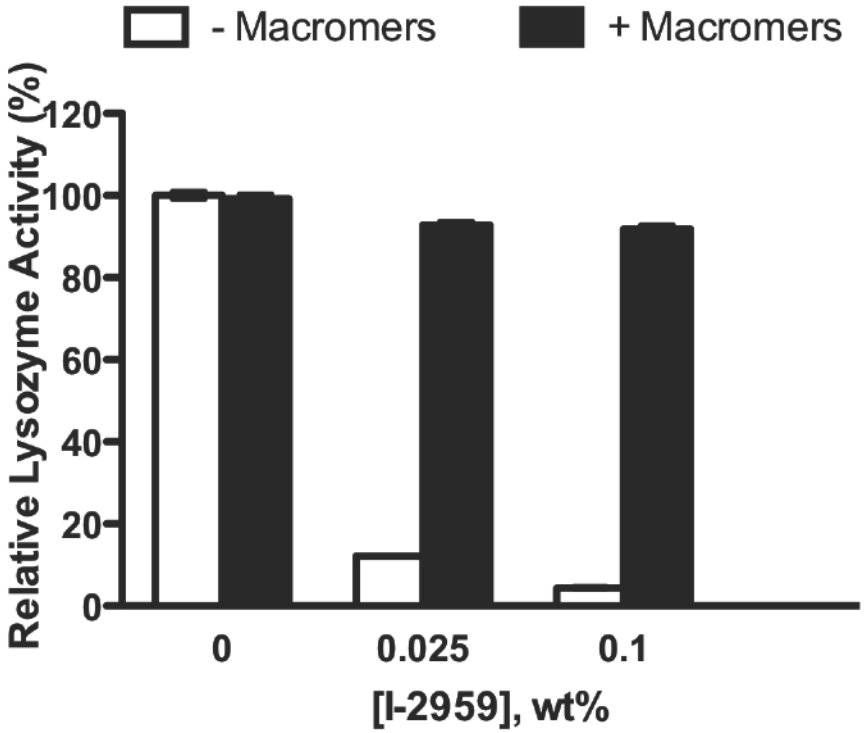

Fig. 7.

Influence of thiol-ene photopolymerization on the bioactivity of lysozyme in the presence of varying concentrations of photoinitiator, I-2959. All samples were exposed to 365 nm light for 10 minutes with or without macromers present in the prepolymer solution.