

Visible light cured thiol-acrylate hydrogels with tunable degradation for controlled protein delivery

Yiting Hao and Chien-Chi Lin

Department of Biomedical Engineering, Purdue School of Engineering and Technology,
Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202, USA

Statement of Purpose: Hydrogels are being investigated for many biomedical applications because of their hydrophilic property and tissue-like elasticity. Hydrogels can be fabricated through photopolymerization using either ultraviolet (UV) or visible light. Visible light initiated photopolymerization is an attractive method for hydrogel preparation. However, traditional visible light-mediated gelation often requires the use of high concentration of triethanolamine (TEA) as a co-initiator.^[1] Adapted from a previously reported visible light initiated thiol-norbornene gelation system,^[2] we report here a highly tunable thiol-acrylate gelation system for fabricating degradable hydrogels. The current gelation system differs from prior acrylate-based visible light mediated photopolymerization in several aspects: (1) co-initiator TEA used in the previous system^[1] was replaced with multifunctional thiols, (2) thiol-acrylate gelation proceeds in a mixed-mode polymerization that yielded gels with less network heterogeneity,^[3] and (3) thiol-acrylate hydrogels can be rendered hydrolytically degradable without the need to synthesize macromers with different degradability. We also utilized the high tunability of thiol-acrylate hydrogel degradation for sustained protein delivery.

Methods: Linear or 4-arm poly(ethylene glycol)-acrylate (PEGDA or PEG4A) and di-thiol containing cross-linkers (e.g., dithiothreitol or DTT) were used to form hydrogels via a visible light mediated mixed-mode photopolymerization. Eosin-Y (0.1 mM) was used as the photoinitiator while NVP (0.1-1.0 vol %) was used as the co-monomer. Gelling kinetics, gel points, and elastic moduli of the thiol-acrylate hydrogels were characterized by photorheometry. Hydrogel degradation was monitored by tracking the changes in gel elastic modulus as time. For protein delivery, bovine serum albumin (BSA, 5 mg/mL) was mixed in the precursor solution and was encapsulated in hydrogels after 5-min of visible light exposure. Bicinchoninic acid (BCA) assay was used to determine the concentration of the released protein.

Results: The mechanism of this visible light initiated gelation is a mixed-mode photopolymerization.^[3] Mechanistically, visible light-excited eosin-Y extracts hydrogen atoms from thiols to form thiyl radicals that propagate through acrylates to form carbon radicals. These carbon radicals either terminate via thiol chain transfer or homopolymerize with other acrylates. This polymerization is a combination of both chain-growth and step-growth photopolymerizations. Figure 1 shows the gelation kinetics of a thiol-acrylate hydrogel (PEG4A-DTT). Gelation occurred ~ 1 min after visible light initiation. We also found that increasing the concentration of the co-monomer NVP accelerates gelation, leads to a gel with a higher elastic modulus and slower hydrolytic degradation. Further, we fabricated non-degradable or

degradable hydrogels by using PEG-tetra-acrylamide or PEG-tetra-acrylate as the macromer and DTT as the cross-linker. Degradation rate could also be tuned by changing cross-linker (e.g., DTT) or NVP concentrations. As shown in Figure 2A, higher DTT concentrations created higher content of thiol-ether-ester bonds following thiol-acrylate gelation, thus leading to faster degradation rates. We also adjusted the gel formulation for degradation-mediated sustained protein delivery. PEGDA (3.4kDa, 15wt%), DTT (30-50 mM), and NVP (0.3%) were used to fabricate degradable hydrogels with small mesh size (6-8 nm). These gels degraded slowly and the degradation rate was proportional to the thiol concentration in the pre-polymer solution. We also found that all gel formulation used in the protein delivery study afforded minimal burst release and increasing thiol content increased the amount of protein release, likely due to enhanced hydrolytic degradation (Figure 2B).

Figure 1. In situ photorheometry of visible light initiated thiol-acrylate photopolymerization (4wt% PEG4A, 4mM DTT, and 1% NVP).

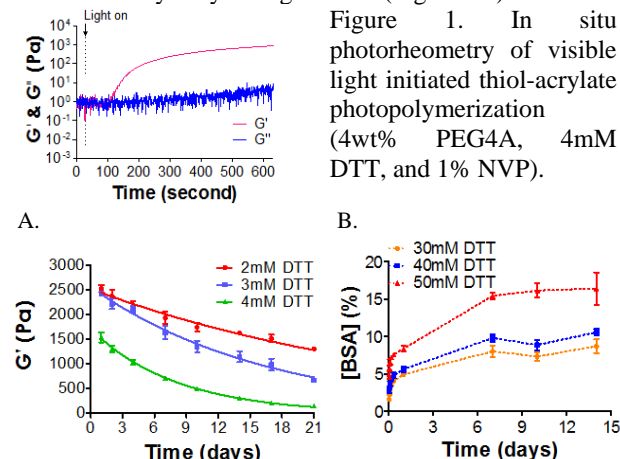


Figure 2. Effect of cross-linker (DTT) concentration on: (A) hydrolytic degradation of thiol-acrylate hydrogels (4wt% PEG4A, 0.1% NVP) and (B) sustained release of BSA from degradable gel (15wt% PEGDA, 0.3% NVP).

Conclusions: In summary, we have developed a versatile visible light-cured thiol-acrylate hydrogels with high tunability in gelation and degradation. Gel degradation rate was tuned by adjusting DTT concentration. The thiol content in the gel formulations also affected the rate of protein release. The use of multifunctional thiols as a co-initiator/cross-linker expands the utility of this gelation system in tissue engineering and controlled protein delivery applications.

References:

- [1] Cruise et al. *Biotechnol Bioeng* 1998; 57: 655-665.
- [2] Shih & Lin. *Macromol. Rapid Comm.* 2013;34:269-273
- [3] Salinas & Anseth *Macromolecules* 2008; 41:6019-6026