

Fundamental Studies of Degradable Thiol-Acrylate Photopolymeric Biomaterials as Tissue Engineering and Drug Delivery Scaffolds

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Introduction The design of new biomaterials for tissue engineering and drug delivery applications is a complex process. Biocompatibility of the material with surrounding tissue, implantation procedures, and function of the material during use must all be considered. In many situations degradable biomaterials that rapidly form *in vivo* under physiological conditions are desirable. Injectable, *in vivo* curing materials minimize implantation difficulty and easily fill complex defects. *In situ* degradation aids repair of damaged or diseased tissue, allows sustained release of therapeutics, and eliminates retrieval procedures. The use of synthetic polymers enables tuning of the biomaterial's physical properties to mimic the surrounding native tissue while controlling the material's degradation behavior. Swollen, polymeric hydrogels have been exclusively investigated as model materials for sustained drug release and host scaffolds for regeneration¹⁻³ of soft tissue, such as cartilage¹⁻³. In hydrogels material properties and degradation behavior are significantly impacted by both the network chemistry and connectivity, which, in turn, are controlled by monomer chemistry, polymerization conditions, and the polymerization mechanism used to form the networks. The impact of network connectivity on degradation behavior in thiol-acrylate materials and the ability to control connectivity in these systems is the focus of this investigation.

Unlike the majority of covalently crosslinked polymer networks which form through chain-growth (**Fig. 1a**) or step-growth (**Fig. 1b**) mechanisms, radically-initiated thiol-acrylate photopolymerizations occur through a combination of step and chain growth mechanisms (**Fig. 1c**). The propagation steps of radically-initiated thiol-ene step-growth and vinyl chain-growth polymerizations are shown in **steps 1-2** and **step 3**, respectively. In thiol-acrylate polymerizations, all three steps occur⁴. The unique thiol-acrylate molecular structure evolves from this mechanism and is directly impacted by thiol:acrylate ratios, transitioning from being more chain-like to more step-like as the ratio of thiol to acrylate groups increases and shortening the kinetic backbone chains generated during polymerization.

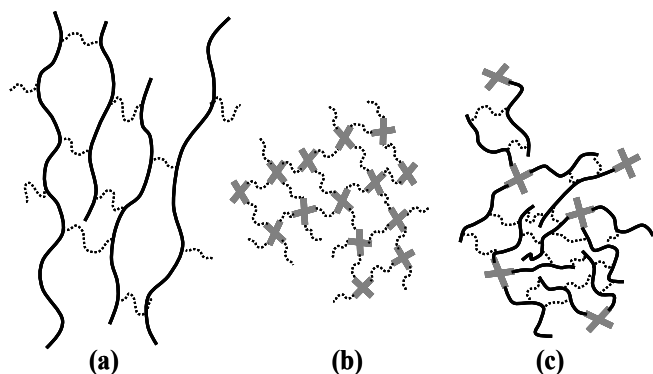
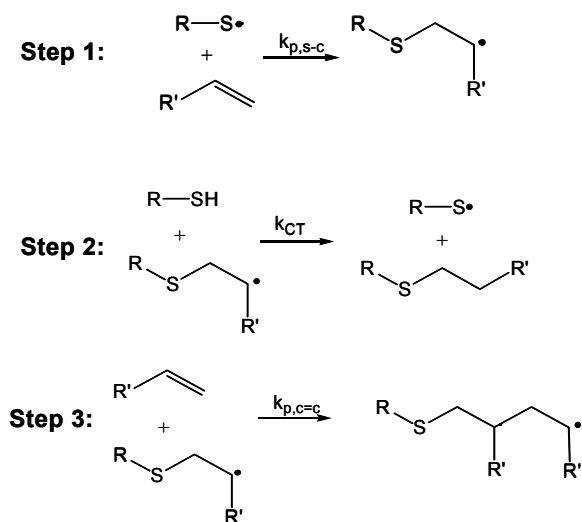


Figure 1: Ideal network connectivity in crosslinked polymers formed from a) chain-growth, b) step-growth, and c) mixed-mode polymerization mechanisms. In each network, the solid black lines represent the kinetic backbone chains, the dotted lines are degradable crosslinks, and the gray X's are multifunctional thiol molecules



Additional advantages of degradable thiol-acrylate biomaterials include rapid polymerization upon exposure to UV light, affording spatial and temporal reaction control. Thiol-acrylate photopolymerization also occur without added photoinitiator, allowing samples with thicknesses well in excess of 10 cm to be formed. Finally, incomplete conversion of all the thiol groups on multifunctional thiol monomers creates tethered sites throughout the network that are modifiable post polymerization.

Experimental The degradable acrylate monomer with three poly(lactic acid) (PLA) units on each side was prepared from poly(ethylene glycol) (PEG), $\overline{M}_n=2000$, using techniques similar to those previously described⁵. Mixtures of degradable diacrylate, various thiol monomers containing one, two or four thiol groups, 50 wt% dimethyl sulfoxide (DMSO), and 0.1 wt% 1-[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one, a photoinitiator, were pipetted into disk-shaped molds and polymerized via exposure to 365 nm light for three hours. The disks were placed into phosphate buffered saline solution (PBS, pH=7.4) in glass jars on an incubated orbital shaker (37°C, 60 rpm). At regular time intervals two to three disks were removed and their mass loss, equilibrium swelling ratios, and compressive moduli were determined.

Samples were prepared without solvent or initiator to investigate both the reaction kinetics in the absence of added photoinitiator and attainable cure depths for these degradable materials. Acrylate conversions were determined using changes in peak area during exposure to UV light for the mid-IR double peak at 1636 and 1620 cm^{-1} or the near-IR peak at 6165 cm^{-1} . To demonstrate the presence of modifiable thiol groups remaining throughout the crosslinked networks, disks made without DMSO were swollen in ethanol to remove any soluble monomer and incubated with Ellman's reagent at room temperature in PBS (pH 8.0).

Results The mechanism involved in thiol-acrylate photopolymerizations was verified through observations of the thiol and acrylate peak areas in mid-IR⁶. The effect of the reaction mechanism on network connectivity and the resulting degradation behavior are shown in **Fig. 2**. The ability of degradable thiol-acrylate mixtures to photopolymerize without added photoinitiator is demonstrated in **Fig. 3**. Controlling the number of pendant thiols available for post-polymerization modification will also be discussed.

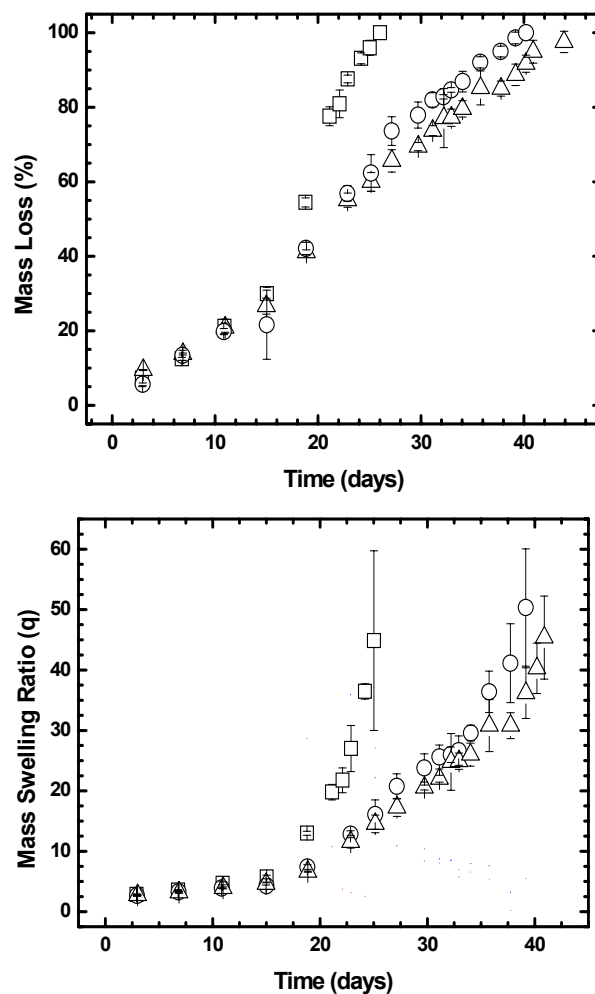


Figure 2: Mass loss and swelling behavior of degradable diacrylate-tetrathiol disks containing 30 (\square), 15 (\circ), and 5 (Δ) mol% thiol groups. Changes in initial thiol concentration shift network connectivity from more step-like to more chain-like, significantly impacting mass loss profiles and swelling ratios. These effects become more noticeable as degradation progresses. Error bars represent standard deviations of three replicates.

Discussion At early time points, neither the percent mass loss nor the equilibrium mass swelling ratios measured for the networks in **Fig. 2** show significant dependence on initial thiol concentration resulting from relatively identical crosslinking densities and similar PLA hydrolysis rates. As degradation proceeds, the effect of initial thiol concentration on network connectivity and backbone kinetic chain length is apparent: networks with higher initial thiol concentrations have shorter backbone chains and fewer crosslinks connecting them to the rest of the network.

Fig. 3 depicts initiatorless thiol-acrylate photopolymerizations. In contrast to the homopolymerization of the PEG-PLA-diacrylate monomer that only reaches 10% conversion in ten minutes, samples containing 15 mol% tetrathiol reach 85% conversion in the same amount of time

Unreacted thiol groups tethered throughout thiol-acrylate photopolymers are a byproduct of the mixed mode mechanism occurring during network formation. The density of potential derivatization sites throughout the polymerized hydrogel is easily controlled by changing the thiol concentration in the initial thiol-acrylate monomer mixture. To demonstrate control over the tethered thiol group concentration in these materials, polymer disks with different initial thiol ratios were exposed to Ellman's reagent (DNTB) in pH 8.0 PBS and the absorbance of the solution was measured. As the initial thiol monomer concentration increased, the solution absorbance also increased, corresponding to an increasing number of tethered, reactive thiol groups in the polymerized networks.

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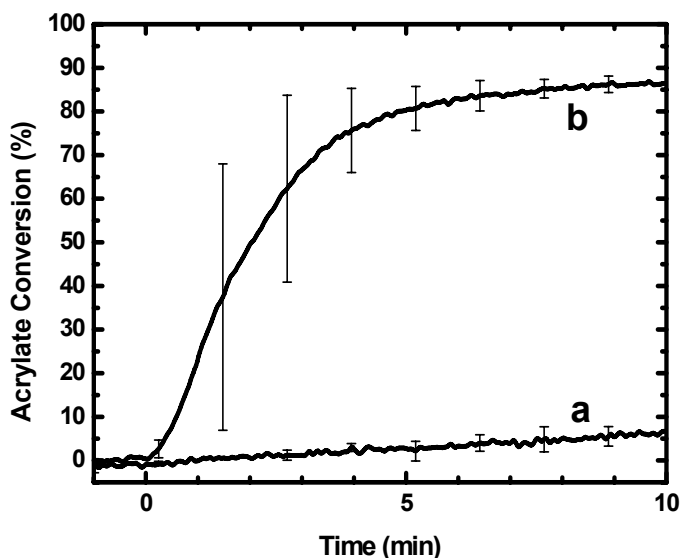


Figure 3: Conversion profiles of initiatorless PEG-PLA-diacrylate photopolymerizations with: a) nothiol and 5 mW/cm² of UV light, b) 15 mol% tetrathiol and 50 mW/cm² of UV light. Error bars represent standard deviations of 2 replicates.