# Enzyme-Responsive, Thiol-ene Hydrogels for Local Therapeutic Delivery at Sites of Inflammation

Alex A. Aimetti[1], Alexandra J. Machen[1], Kristi S. Anseth[1,2]
[1]Department of Chemical and Biological Engineering and the [2]Howard Hughes Medical Institute
University of Colorado
UCB 424
Boulder, CO 80309-0424

**Statement of Purpose:** Responsive biomaterials that demonstrate both spatial and temporal control have potential application in a variety of biomedical applications. In particular, enzyme-responsive biomaterials can be designed to locally delivery therapeutics in recognition of a specific cellular response, such as inflammation. Within inflammatory microenvironments, activated neutrophils degranulate releasing a variety of enzymes including human neutrophil elastase (HNE). As such, we sought to utilize this inflammatory-based response as a way to trigger local delivery of biologically relevant therapeutics from poly(ethylene glycol) (PEG)-based hydrogels. Thiol-ene photochemistry[1] was utilized as a facile method to fabricate PEG hydrogels with enzyme sensitive peptide cross-links rendering the material degradable upon exposure to HNE. In addition, the tunable-release of physically-entrapped therapeutics is directly related to the rate of gel erosion.

**Methods:** Four-arm PEG tetra-norbornene, bis-cysteine HNE-cleavable peptide[2], and photoinitiator were combined in a monomer solution. Upon exposure to UV light, thiyl radicals form that subsequently add to the norbornene functionality in a stepwise fashion ultimately resulting in a three-dimensional, cross-linked network. HNE-dictated cleavage occurs between the P1 and P1' amino acid positions (Figure 1). PEG molecular weights ($M_n$=5,000 and 20,000) and peptide linkers (CGAAPV↓RGGGGC & CGAAP(Nva)↓GGGGGC) were varied to study their influence on degradation kinetics. To characterize erosion, HNE was exogenously added at physiological concentrations (2μM-200nM) and the hydrogel fractional mass loss was determined. Bovine serum albumin (BSA) was encapsulated as a model therapeutic within the hydrogel and its release monitored in response to HNE treatment.

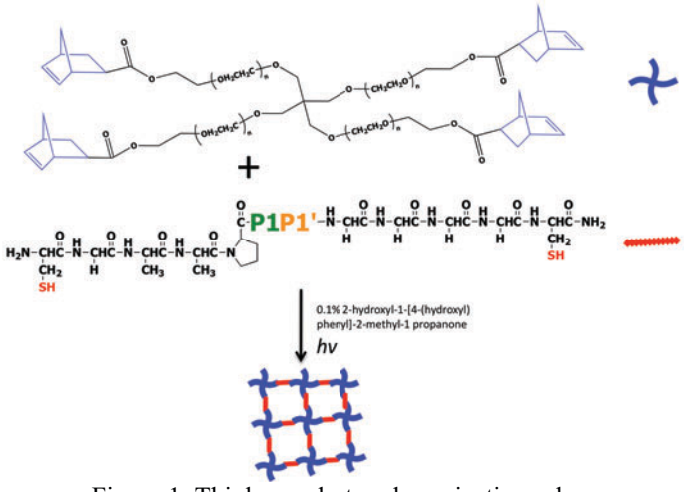


Figure 1. Thiol-ene photopolymerization scheme

**Results:** Hydrogel degradation kinetics proved to be dependent on PEG molecular weight, peptide cross-link, and concentration of HNE. At increased enzyme reaction rates, gel degradation is surface mediated. However, as the rate of reaction decreases (i.e. decreased [HNE]), the mass loss profile begins to deviate from a surface eroding system (Figure 2A). In addition, previous work in the lab has shown the peptides AAPV↓RGCG and AAP(Nva)↓GGCG exhibit HNE cleavable rate constants ($k_{cat}$) of 3.13 $s^{-1}$ and 0.56 $s^{-1}$, respectively. By incorporating the peptide cross-link with the norvaline (Nva) residue, we observed a decrease in gel degradation rates. Therapeutic delivery profiles were in agreement with the mass loss profiles under the same external conditions and gel compositions (Figure 2B).

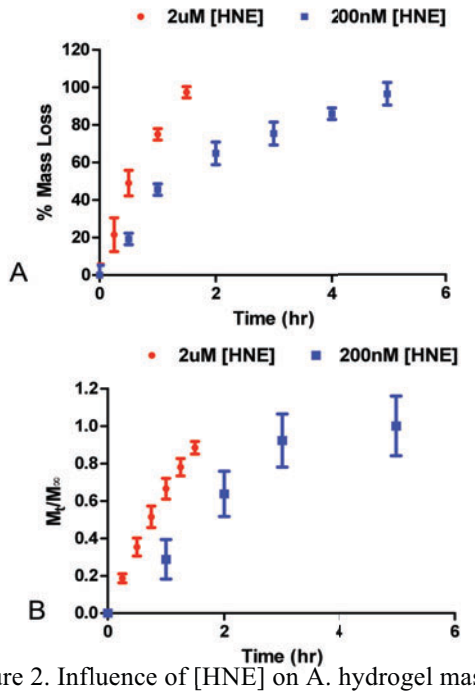


Figure 2. Influence of [HNE] on A. hydrogel mass loss and B. BSA release from gel

**Conclusions:** This work aims at developing a novel, environmentally-responsive drug delivery hydrogel capable of releasing a therapeutic at specific sites of inflammat ion. Thiol-ene photopolymerization is used as an easy way to incorporate synthetic peptide cross-links into the network. By engineering different synthetic cross-links and gel formulations, a tailorable therapeutic release profile was achieved that can be exploited for localized delivery *in vivo*.

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**References:**

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