**Redox-Responsive Hydrogels with Decoupled Initial Stiffness and Degradation**

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*Statement of Purpose:* Creating hydrogels with tunable properties (e.g., mechanical or degradation properties) is desirable for tailoring hydrogel formulations for specific applications. In this work, we developed a poly(ethylene) glycol (PEG) hydrogel with decoupled initial stiffness and degradation. The hydrogel was formed in a one-pot cross-linking method that formed both thioether and disulfide bonds via divinyl sulfone (DVS) and $Fe(EDTA)_3$. By varying the amount of DVS, the ratio of nonreducible thioether bonds to reducible disulfide bonds was adjusted to allow for different degradation profiles while keeping storage modulus fairly constant. Gels had tunable release rates of dextran and were cytocompatible.

*Methods:* DVS from a stock solution at 80 mM in phosphate buffer saline (PBS) was first combined with Fe-EDTA (100 mM ferric ion, 110 mM EDTA, PBS, pH = 7.4). PEG-SH at 12 wt% in PBS (pH 7.4) was added and resulted in 5 wt% PEG; 50 mM ferric ion; and 0, 2, 4, 6, or 8 mM DVS. Rheological characterization was performed on an AR-G2 rheometer (TA Instruments, New Castle, DE) using a 20-mm plate geometry. Compression testing was performed on a BOSE dynamic mechanical analyzer (Framingham, MA), with elastic modulus calculated from the slope of the stress-strain curve of the second cycle of an oscillatory run. Degradation and swelling studies were performed after gels polymerized for 24 h at 37 °C, and gels were incubated in 1 mL of PBS with or without 10 $\mu$M GSH. Two molecular weights of FITC-dextran (150 kDa and 2 MDa) were encapsulated in gels, and release profiles over 120 h were evaluated in both PBS and PBS with GSH. Cytocompatibility was determined via a viability assay after 1 and 5 days post-encapsulation.

*Results:* PEG hydrogels were created with both thioether and disulfide bonds via crosslinking with DVS and reacting with Fe(EDTA), respectively. All gel formulations underwent bulk polymerization within ten minutes as determined by inversion tests and exhibited gelation times of 2-8 minutes via rheological time sweeps. Mechanical properties were assessed via rheological strain and frequency sweeps to determine storage moduli and via compression testing to measure elastic moduli of the hydrogels (Figure 1). Storage moduli of 0 mM and 2 mM DVS hydrogels were statistically lower than 4 mM DVS hydrogels, and 8 mM DVS hydrogels resulted in moduli similar to both groups (Figure 1A). Elastic moduli were statistically higher for 2 mM and 4 mM DVS gels compared to 0, 2, and 8 mM, which were all statistically similar (Figure 1B).

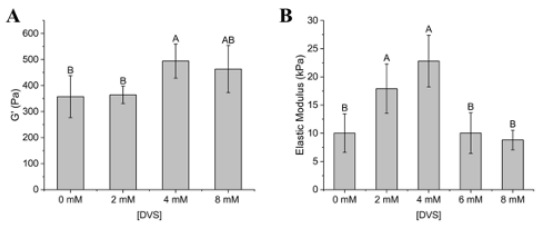


Figure 1. Mechanical properties of hydrogels: (A) storage moduli (G') and (B) elastic moduli. Groups with identical letters are statistically similar ($p > 0.05$).[1]

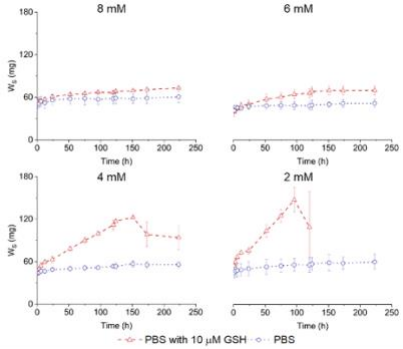


Figure 2. Degradation profiles of gels in 10 $\mu$M GSH or a PBS control expressed as wet weight ($W_s$) over time.[1]

Degradation profiles in the presence of GSH were highly dependent on the DVS concentration, or the relative amount of thioether bonds relative to reducible disulfide bonds (Figure 2). For gels with 6 or 8 mM DVS, wet weights remained stable with or without GSH. Gels with 2 and 4 mM DVS both exhibited increases in wet weight when swelled with GSH, reaching peaks after 96 h and 175 h, respectively. The gels with 2 mM DVS in GSH completely degraded after 120 h. GSH had a similar impact on FITC-dextran release profiles for 2 MDa dextran. The differences in cumulative release after 120 h of incubation between gels in PBS with GSH to PBS alone were 45%, 27%, 13%, and 2.1%, for 2, 4, 6, and 8 mM DVS gels, respectively. Nearly all the encapsulated cells were alive after 1 and 5 days in gels.

*Conclusions:* In this work, 4-arm thiolated PEG was reacted with Fe(EDTA) and crosslinked with varying amounts of DVS to form both reducible disulfide bonds and non-reducible thioether bonds. By varying the amount of DVS, initial stiffness was decoupled from degradation rates. Swelling and dextran release profiles were modulated by adjusting the ratio between thioether bonds and disulfide bonds. Our findings suggest that our hydrogel system is a promising platform for redox-responsive drug delivery or tissue engineering applications.

*Reference*: [1]Lin, CY. Biomacromolecules. 2021; *accepted*