Amplified Photodegradation of Cell-laden Hydrogels Through an Addition-Fragmentation Chain Transfer Reaction

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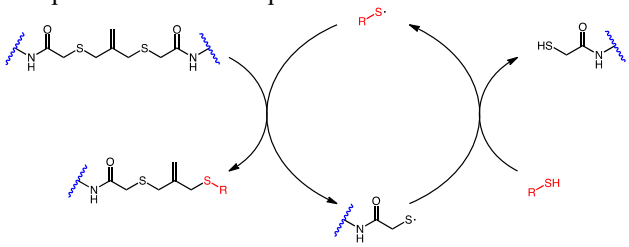
Statement of Purpose: Hydrogels that can be manipulated through photochemical reactions have become a valuable tool to create dynamic cell scaffolds. Such light-responsive platforms allow researchers to manipulate the cell environment with spatial and temporal control. In particular, photodegradable hydrogels have been used to create local variations in mechanics and topography to investigate cellular processes. While significant strides have been made, current cytocompatible photodegradation relies on slow alpha cleavage of photoactive species incorporated into polymer chains (i.e. "one-photon, one-event" processes). We have improved upon this platform by crosslinking cell-laden networks with allyl sulfide chain transfer agents. Exposure of these scaffolds to light in the presence of a photoinitiator and free monofunctional thiol results in addition-fragmentation chain transfer reactions that rapidly replace crosslinking allyl sulfides with their non-crosslinking counterparts (Scheme 1). The photodegradation kinetics of this reaction are far superior to those of backbone alpha-cleavage. Furthermore, a lower concentration of photoactive species is employed, enabling the degradation of much thicker samples. This reaction also proceeds in a cytocompatible manner, and primary human mesenchymal stem cells (hMSCs) remain viable through encapsulation and subsequent release.



Scheme 1: Amplification of photodegradation by alternating cycles of thiol addition to olefin of allyl sulfide and chain transfer to soluble thiol.

Methods: Hydrogels were formed by crosslinking dibenzocyclooctyne-terminated poly (ethylene glycol) (PEG-DBCO) with an azide-flanked symmetric allyl sulfide through a strain-promoted azide alkyne cycloaddition. For photodegradation, heterobifunctional methoxy-PEG-thiol (mPEG-SH, 0-50 mM) and photoinitiator lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate (LAP, 2-8 mM) were diffused into the hydrogel, followed by exposure to 365 nm light (2-40 mW cm^{-2}). Gelation and photodegradation were monitored by rheology, and the gel point and reverse gel point were estimated by the crossover of the storage (G') and loss (G") moduli. hMSCs were encapsulated within the hydrogels and cultured under standard conditions. Viability was assessed using calcein AM/ ethidium homodimer staining. Prior to light exposure, LAP and mPEG-SH were diffused into the gel for 1 h.

Results: Hydrogels crosslinked by allyl sulfide-containing molecules were found to undergo extremely rapid photodegradation. This rate was characterized as a function of mPEG-SH concentration (Figure 1a). Increasing the concentration of soluble monothiol was found to greatly enhance degradation rate, consistent with the hypothesis that chain transfer to free thiols and subsequent addition to allyl sulfides results in an amplification of the photodegradation reaction. Indeed, if we define a "quantum yield" for this reaction as the number of crosslinks cleaved per photon absorbed, this value is greater than unity. The photodegradation rate also increases with photoinitiator concentration and light intensity (data not shown). Normalized with respect to light dose, the kinetics of this reaction are 2-3 orders of magnitude higher than those seen with backbone alpha-cleavage hydrogels. Furthermore, a lower concentration of photoactive species is employed, allowing for degradation of much thicker samples. Whereas current cell-laden photodegradable hydrogels are limited in practice to the top ~100 μm, we demonstrate the reverse gelation of a 1 cm thick gel (Figure 1 b-c). Importantly, the reaction is also mild enough to preserve primary cells, as hMSCs remain viable in the hydrogel and through subsequent release (Figure 1 d-e).

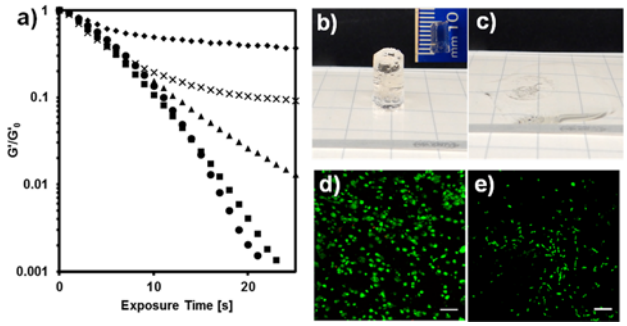


Figure 1. Rapid, cytocompatible photodegradation of hydrogels. a) Rheological trace showing normalized storage modulus as a function of irradiation time. Hydrogels were swollen with 4 mM LAP and 0 (◆), 5 (×), 15 (▲), 25 (■), or 50 mM (●) mPEG-SH. b) 1 cm tall hydrogel before and after (c) light exposure for 74 s. d) hMSCs remain viable through encapsulation. Scale bar 100 μm. e) Released cells are viable and spread over 24 h after release onto a coverslip. Scale bar 300 μm.

Conclusions: We have developed hydrogels that are degradable through a photoinitiated radical reaction. This strategy results in cytocompatible photodegradation that is much faster, and penetrates much deeper, than has previously been reported in biological applications. This platform should greatly extend the utility of light-responsive hydrogels as tunable cell scaffolds, and also opens the door for many applications that have previously been precluded by light dose or attenuation.