Dithiolane-Based Dynamic Hydrogels for Photoinduced Crosslinking, Exchange, and Depolymerization

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Statement of Purpose: Advances in the development of adaptable and responsive hydrogel chemistries that are compatible with cell culture have allowed researchers to study the effects of dynamic cell-matrix interactions on many cellular processes. Because signaling events and mechanical transitions in and around cells vary in time and space, stimuli-responsive dynamic covalent chemistries (DCCs) have been increasingly incorporated into hydrogels that are used for 2D and 3D cell culture, enabling user-dictated manipulation of mechanical or biochemical characteristics.[1] Many hydrogel chemistries have been developed for individual stimuli such as light, pH and temperature, and responses including stiffening, softening, and induced viscoelasticity. However, few single-component systems are capable of reversible, repeatable, or even opposing transformations in network structure and functionality. We sought to design a multi-responsive single-chemistry crosslinker for hydrogel cell culture platforms using 1,2-dithiolanes, which are inherently photoreactive ring strained disulfides capable of initiator-free photopolymerization.[2] The resultant materials contain intrinsically dynamic disulfide crosslinks capable of responding to multiple user-controlled stimuli.

Methods: We first prepared synthetic poly(ethylene glycol) (PEG) and hyaluronic acid (HA) macromolecules functionalized with lipoic acid, a common 1,2 dithiolane. Using a photo-rheometer, we characterized the *in situ* gelation of these hydrogels through the initiator-free ring-opening crosslinking of the pendant dithiolanes (Figure 1A). By modifying the composition of the gels or swelling solution, we demonstrate a series of different photoinduced responses to manipulate gel mechanics and characterize each action through rheology. Applications of these unique material properties are highlighted through a series of cell experiments conducted in 2D and 3D using both primary cells (human mesenchymal stem cells, hMSCs) and a cell line (C2C12 myoblasts).

Results: Photorheology results demonstrated efficient initiator-free crosslinking of dithiolane functionalized hydrogel precursors using 365 nm light, resulting in fast crossover at cytocompatible light doses (within 120 s at 5 mW/cm^2, Figure 1B). Using low light doses, hMSCs were encapsulated in both PEG-lipoic acid (PEG-LA) and HA-lipoic acid (HA-LA) gels while maintaining high viability (>90%, Figure 1C). Because of the disulfide crosslinks, this system has dynamic linkages post gelation, enabling conjugation of ligands, light induced changes in viscoelasticity, photosoftening of the modulus, and other controllable material properties. In one example, we used a lipoic acid functionalized RGD peptide to photopattern localized adhesive ligands that promote C2C12 attachment and spreading, and then employed the radical-mediated photosoftening mechanism to release adhered C2C12s in a spatially defined manner (Figure 1D). Finally, we fabricated PEG-LA hydrogels into various complex 3D structures, including topographically patterned microwells and electrosprayed microgels. Collectively, these results show enhanced control over material properties using LA crosslinked hydrogels, including stiffness, geometry, composition, and viscoelasticity.

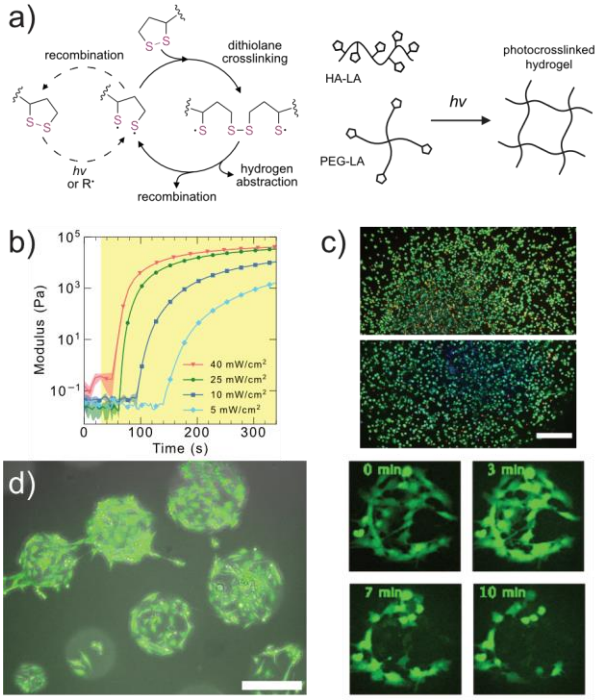

Figure 1. Dithiolane crosslinking for hydrogel formation and dynamic cell culture. a) Dithiolanes undergo a photoinduced ring-opening polymerization to form hydrogel networks. b) Fast gelation is observed using 365 nm light at various light intensities. c) hMSCs demonstrated very high (> 90%) viability on day three of culture in PEG-LA (top) and HA-LA (bottom) gels. d) C2C12s adhere with high selectivity to photopatterned LA-RGD. Using photoinitiator swelled into the network, cells were released with patterned 405 nm light from a confocal microscope and imaged over the course of 20 minutes as cells retracted. Scale bars = 300 μm.

Conclusions: Lipoic acid crosslinked hydrogels allow for the tailoring of a diverse array of material properties with adaptive responses to exogenous stimuli. Our experiments demonstrate several biomaterials applications where this single hydrogel chemistry provides unique benefits for studying and directing cell-material interactions.

References: [1]Qazi, T., et al., *Cell Stem Cell* 2022. [2]Barltrop, J., et al., *JACS* 1954.

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