Tuning Hydrogel Mechanics with Dynamic Covalent Chemistry

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Statement of Purpose: Cell phenotype and behavior are influenced by mechanical information from the extracellular matrix (ECM). Tissues exhibit two mechanical properties of particular interest: stiffness and stress relaxation. Profound insights into cell behavior have been enabled by hydrogels that replicate these features. Recognizing the need for synthetic matrices that exhibit more ECM-mimetic properties, researchers have turned to incorporating dynamic chemistries in hydrogel crosslinks. In these systems, stiffness is controlled through crosslink density, and reversible crosslinking imparts stress relaxing properties. However, the effects of matrix stiffness and stress relaxation on cell behavior in these gels remain challenging to decouple. Here, we report on two efforts to 1) develop a hydrogel with independently tunable mechanical properties by designing crosslinkers that engage associative thiol exchange chemistries[1] and 2) apply a visible-light-responsive boronic ester-based hydrogel with reversible mechanics to interrogate behavior of D1 mesenchymal stem cells.[2] The former illustrates how structure-reactivity relationships gleaned from kinetics of crosslinker exchange directly allows us to control the mechanical properties of hydrogels. The latter demonstrates how incorporating a photoswitchable azobenzene proximal to a dynamic crosslink provides a handle to interrogate cell mechanobiology with spatiotemporal control. These hydrogels contribute a means to study unexplored biomechanical mechanisms underlying physiology and disease.

Methods: *Developing hydrogel system with associative crosslinkers.* The kinetics of associative thiol exchange for dithiol alkylidene crosslinkers were characterized by obtaining second order rate constants in small molecule systems. Hydrogels containing crosslinkers were fabricated *in situ* on a rheometer through thiol-ene chemistry. Stress relaxation experiments were performed, and time scales of stress relaxation were acquired by fitting normalized data to single-element Maxwell models. *Interrogating cell behavior using a photoswitchable dynamic hydrogel with reversibly control mechanics.* Poly(ethylene glycol) (PEG) polymers were functionalized with terminal azobenzene boronic acid and azide end groups (PEG-Azo-Azide), gluconolactone and azide end groups (PEG-Glu-Azide), and dibenzocyclooctyne (PEG-DBCO). D1 murine mesenchymal stem cells (MSCs) were encapsulated in 1:1:1 10 wt% 8-arm 10 wt% PEG gels containing 1 mg/mL type I collagen. Cells were subjected to three treatment conditions: 1) cultured in the dark for 6 hours, 2) exposed to 10 minutes of green light (λ=530 nm) at time 0, and 3) exposed to 10 minutes of green light at time 0, followed by 3 minutes of blue light (λ=470 nm) after 3 hours. Cell volume and sphericity were quantified, and gene expression was monitored using qPCR.

Results: *Developing a hydrogel system with tunable stress relaxation and constant stiffness.* Thiol exchange rates for dithiol alkylidenes were shown to span over three orders of magnitude, with k_{ex} of 6.99 and 3.77 x 10^{-3} $M^{-1}s^{-1}$ for the fastest cyclic and slowest linear structures respectively. In hydrogels, the reactivity of the crosslinkers translated to polymer stress relaxation: the fastest exchanging crosslinkers exhibited stress relaxation on the order of 10^{-1} s whereas the slowest ones relaxed stress on the order of 10^3 s. These results corroborate that stiffness and stress relaxation can be independently tuned using crosslinks that exchange through an associative mechanism. *Interrogating cell behavior using a photoswitchable dynamic hydrogel with reversibly controlled stiffness.* D1 MSCs exposed to green light exhibited isotropic spreading with a significant increase in cell volume, but not cell sphericity (Figure 1). Increases in *bactin*, *acta2*, *ankrd1*, and *ctgf* gene expression implicate actomyosin contractility as a potential mediator of mechanosensing. Cells that were further exposed to blue light exhibited isotropic shrinking. These results demonstrate the utility of photoresponsive dynamic covalent hydrogels as a tool to interrogate cellular mechanosensing with spatiotemporal control.

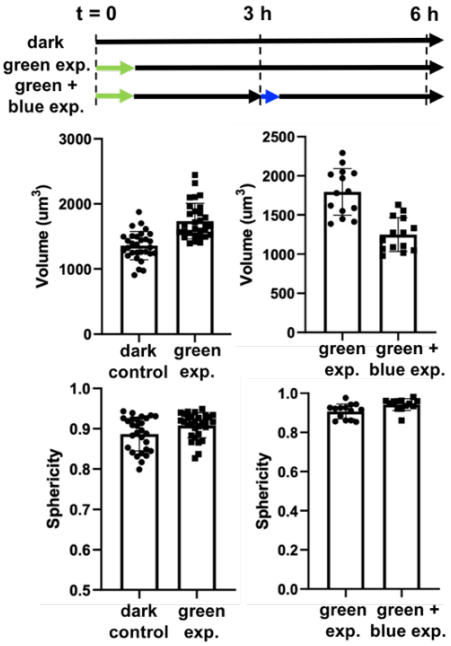

Figure 1. D1 murine MSCs respond to photocontrolled stiffening and softening of their environment. Cells are initially cultured on a soft substrate, which stiffens upon green light exposure and softens upon blue light exposure.

References:

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