

A Strategy for Reversible Control of Hydrogel Modulus to Probe Myofibroblast Activation

Adrianne M. Rosales,¹ Kelly M. Mabry,¹ Christopher B. Rodell,² Jason A. Burdick,² Kristi S. Anseth^{1,3}

¹Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO

²Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania

³Howard Hughes Medical Institute, University of Colorado, Boulder, CO

Statement of Purpose: The extracellular matrix is a dynamic environment that increases in stiffness during fibrosis, leading to myofibroblast activation, and in some cases, subsequent decreases in stiffness upon resolution of the disease [1]. However, many *in vitro* cell culture platforms have static moduli, requiring one to replate cells to study physiologically-relevant changes in stiffness. *In situ* stiffening of the substrate would better simulate the changes observed in disease progression, and ensuing matrix softening would enable investigation of the biological mechanisms that may be involved in disease resolution. However, there are few examples of materials with user-defined, reversible moduli, and existing platforms introduce altered charge and redox states that may influence cell behavior. Here, we present a strategy to reversibly control the modulus of hierarchically-ordered hydrogels with light exposure through the use of an azobenzene photoisomer. This photoisomer can either modulate the structure of a peptide crosslinker in PEG-based hydrogels, or it can reversibly associate with cyclodextrin units in hyaluronic acid (HA)-based gels to modulate crosslinking density (Fig 1A), leading to responsive biomaterials to probe dynamic mechanics.

Methods: A peptide crosslinker containing azobenzene, was synthesized using Fmoc-based solid phase peptide synthesis. The peptide contained cysteines at both termini for incorporation into PEG hydrogels via Michael addition. HA was separately functionalized via amidation with cyclodextrin or esterification with 4-phenylazobenzoic acid, then mixed in a 1:1 ratio to form supramolecular gels. The mechanical properties of the hydrogels were measured using shear rheology with a light accessory for stimulus with either UV (365 nm, 10 mW/cm²) or visible (400–500 nm, 10 mW/cm²) light. Porcine aortic valvular interstitial cells (VICs) were encapsulated into the PEG gels with 2 mM RGD at a density of 10 million cells/mL.

Results: Upon irradiation with UV light, azobenzene isomerizes from a planar *trans* configuration to a bent *cis* configuration (Fig 1A). In the peptide-crosslinked PEG gels, this isomerization breaks hydrogen bonds, thereby destabilizing the peptide structure and leading to an overall softening of the bulk modulus (Fig 1B). Irradiation with visible light (400–500 nm) leads to reverse isomerization from *cis* to *trans*, allowing for the elastic modulus to return to its initial state. Importantly, the azobenzene unit was chosen carefully to maximize the lifetime of the *cis* state (half-life of 9 hours at 37°C or 162 hours at room temperature). As shown in Figure 1B, when the light is off, there is minimal change in the modulus in either the *trans* or the *cis* state. In hydrogels formed by supramolecular assembly, the hydrogels exhibit much more frequency-dependent behavior due to the dynamic nature of the cyclodextrin-azobenzene associative

crosslinks. When azobenzene is in its *trans* conformation, the association constant with β -cyclodextrin is approximately 800 M⁻¹, which is much higher than the association constant with the *cis* isomer (~300 M⁻¹) [2]. As a result, a decrease in both the storage and the loss modulus are seen upon irradiation with UV light (Fig 3C, filled symbols, 10 wt% gel). To demonstrate cytocompatibility with a fibrosis-related cell type, VICs are encapsulated into the azobenzene-containing PEG gels (Fig 1D). After three days, viability is high (>80%), and the cells exhibit a spread morphology.

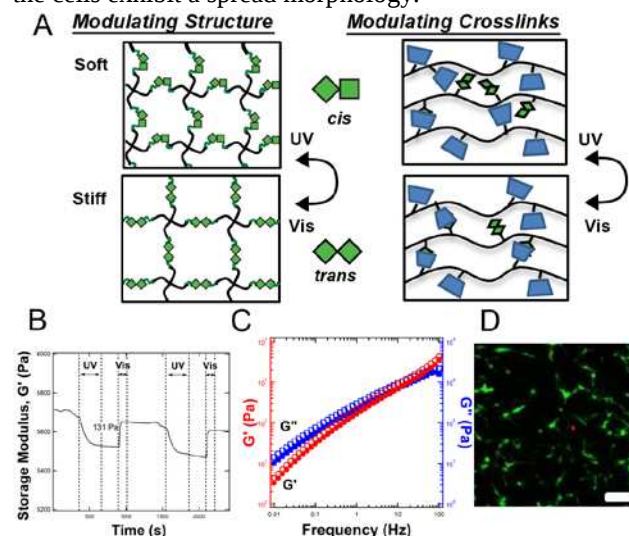


Figure 1. A. Reversible control of hydrogel modulus via use of azobenzene (green diamonds) to modulate peptide crosslinker structure (left) or crosslink density (right) with cyclodextrin (blue trapezoids) complexes. B. *In situ* rheology shows dynamic changes in elastic modulus with UV and visible light for PEG-based gels. C. Both the storage and the loss moduli decrease when the UV light is on (filled symbols) for HA-based supramolecular gels. D. High viability of encapsulated VICs in PEG-based azobenzene gels (green = live, calcein-AM; red = dead, ethidium homodimer; scale bar = 100 μ m).

Conclusions: Incorporating azobenzene into hydrogel crosslinkers in two separate systems allows for the reversible control of gel modulus with light. Future work will focus on tuning the gel properties to span a modulus range relevant to VIC myofibroblast activation in 3D. For example, additional peptide structures or types of cyclodextrins will be considered to increase the impact of isomerization. Due to their tunability and non-invasive stimulus, these innovative materials may be broadly useful for probing the effect of dynamic stiffness on many cell types.

References:

- [1] Hinz, B. *et al. Am J Pathol.* 2012; 180:1340-1355.
- [2] Yamaguchi, H. *et al. Nat. Commun.* 2012; 3:603.