Reversibly Stiffening Hydrogels to Probe Myofibroblast Activation

Adrianne M. Rosales,[1] Eric M. Nehls,[2] Kristi S. Anseth[1,2]
[1] Howard Hughes Medical Institute, University of Colorado, Boulder, CO
[2] Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO 80303

Statement of Purpose: In addition to soluble factors, mechanical forces from the extracellular environment play a critical role in the development of fibrotic-related disease. Until recently, it has been difficult to develop cell culture substrates with stiffness conditions that accurately mimic the progression from healthy to diseased tissue. In particular, many cell culture substrates exhibit a stiffness that is not physiologically relevant, and this can lead to unintended changes in cell phenotype (e.g., myofibroblast activation of fibroblasts). As a result, hydrogels have emerged as a class of cell culture substrates with tunable moduli. For example, poly(ethylene glycol) (PEG) hydrogels can be tuned for valvular interstitial cell (VIC) culture, to span a range of moduli from soft, de-activating conditions (5-10 kPa) to stiff, activating conditions (~25 kPa) [1,2]. However, changes in the elastic moduli of these gels are typically controlled by photodegradation and re-crosslinking reactions, which may lead to undesired changes in network microstructure. In addition, such changes are often irreversible. Here, we present a peptide-crosslinked PEG hydrogel platform with moduli that can be *reversibly* controlled by light exposure. The developed hydrogels will enable noninvasive control of the substrate modulus independent of changes in the chemical composition or network connectivity, allowing us to probe questions about the effect of a dynamic matrix stiffness on the myofibroblast activation process.

Methods: A peptide crosslinker containing azobenzene, a photoisomer, was synthesized using Fmoc-based solid phase peptide synthesis. The peptide was 15 residues in length and contained cysteines at both termini for subsequent incorporation into PEG hydrogels. The absorption properties of the crosslinker in aqueous solution were measured using UV/Vis spectroscopy both before and after irradiation at 365 nm (10 mW/cm^2). The crosslinkers were reacted with norbornene-functionalized multi-arm PEG chains using thiol-ene click chemistry to form the hydrogel substrates.

Results: Azobenzene is often incorporated into peptides as a handle to control chain conformation with light. Upon irradiation with UV light (~320 – 360 nm), azobenzene isomerizes from a planar *trans* configuration to a bent *cis* configuration (Figure 1a), leading to a distance change in the peptide length. This isomerization corresponds to a shift in the peptide's absorption spectra, which can be monitored via UV/Vis spectroscopy as shown in Figure 1b. After irradiation with 365 nm light for 5 minutes at 10 mW/cm^2, the peptide crosslinker developed here shows a strong decrease in the absorption maximum at 325 nm and a slight increase in the maximum around 420 nm. When incorporated into a gel, this *trans*-to-*cis* isomerization induces strain into the network, leading to an increase in the elastic modulus. Importantly, the change in the peptide crosslinker conformation is reversible; dark adaptation enables thermal relaxation of the peptide back to a 100% *trans* state on the timescale of days. Alternatively, the *cis*-to-*trans* isomerization can be induced by irradiation with light at 405 nm, leading to user-controlled manipulation of the gel properties.

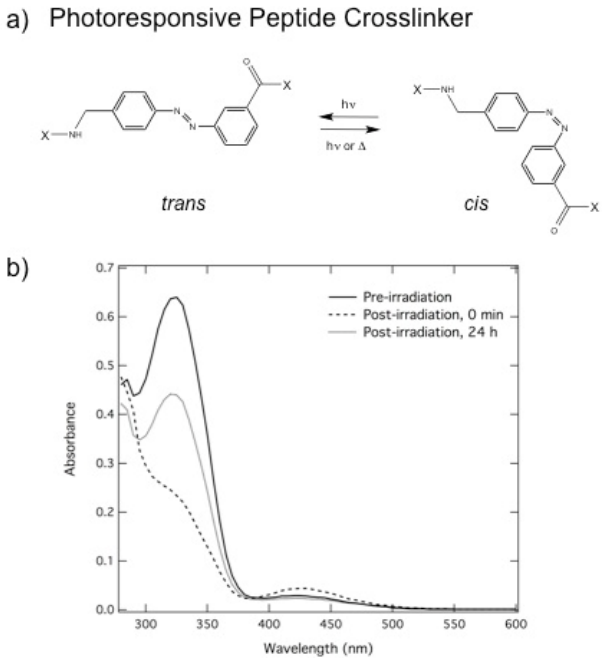

Figure 1. a) Azobenzene moiety incorporated into peptide crosslinker; "X" denotes amino acids. b) Absorbance spectra for peptide crosslinker in 10 mM PBS at 25°C (100 μM). Irradiation occurred at a wavelength of 365 nm for 5 min at 10 mW/cm^2 intensity.

Conclusions: Incorporating azobenzene into a peptide crosslinker allows for reversible control of the chain conformation with light. Within a PEG hydrogel system, the isomerization of these peptide crosslinkers translates to a reversible change in gel mechanical properties. Future work will focus on optimizing gel properties to span a modulus range relevant to VIC myofibroblast activation. Furthermore, VICs will be cultured on the developed substrates and exposed to a temporally controlled range in environmental stiffness by tuning the modulus with predetermined doses of light. Thus, the gel system presented here will enable a unique study on the reversibility of the myofibroblast activation process without invasive changes to the underlying cellular substrate. In addition, these innovative materials may be broadly useful for probing the effect of changing stiffness on other cell types without changes in network connectivity.

References:

[1] Kloxin AM. Biomaterials. 2010; 31(1): 1-8.
[2] Gould ST. Acta Biomaterialia. 2012; 8: 3201-3209.