Dynamic and Reversible Stiffness Modulation in Photoresponsive Protein-Polymer Hydrogels

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Statement of Purpose: There is a growing appreciation for the large role that mechanical signals presented by the local extracellular matrix (ECM) have on cell fate. Through direct interaction with physical cues presented in the cellular ECM, these external mechanical signals are translated into internal biochemical responses that govern gene expression and cell fate decisions[1]. Seminal findings in the field of cellular mechanotransduction have demonstrated that matrix stiffness alone can drive changes in essential processes including attachment, morphology, migration, proliferation, and differentiation. More recently, it has been observed that stem cells possess mechanical "memory", storing information about past physical culture conditions to influence future functions[2]. These findings represent landmark observations that are rapidly changing standard practices in molecular biology and stem cell culture.

Efforts to elucidate the specific effects that ECM elasticity has on cell physiology have been performed almost exclusively on static biomaterial systems. While these studies have provided invaluable insight into the critical roles in which ECM stiffness regulates cellular function and fate, such simple systems fail to recapitulate biophysical dynamics known to accompany tissue/organ development, regeneration, wound healing, and disease progression. Beyond long-term tissue stiffening or softening, cells also experience cyclic loading that periodically and reversibly alter local ECM rigidity; pulsatile flow associated with blood being pumped through the circulatory system places cyclic loads on cells virtually everywhere throughout the body. Unfortunately, materials capable of capturing these sorts of dynamic and reversible stiffening events remain largely undeveloped, making it challenging to understand these essential biological processes[3–7]. This work highlights our efforts to develop new material platforms to mimic the anisotropic and periodic reversible stiffening that occurs *in vivo*. Fundamental knowledge from these studies will lead to newly expanded opportunities in therapeutic tissue regeneration and precision medicine.

Methods: Chemoenzymatic strategies were used to introduce reactive azides onto the N and C termini of photosensitive recombinant proteins. Proteins purity was assessed by whole-protein mass spectrometry. A gel shift assay was utilized to verify successful installation and sustained reactivity of bioorthogonal azide handles. Cell-laden hydrogel networks were formed upon reaction of a four-arm poly(ethylene glycol) tetra(cyclooctyne), a bis(azide) photosensitive protein in the presence of a cell suspension. Upon gel formation, hydrogels were exposed to visible light (I = 10 mW cm^2) to induce on-demand softening. *In situ* rheology was utilized to quantify dynamic moduli changes under light and dark conditions (Figure 1).

Results: We have successfully expressed and purified our photoresponsive recombinant proteins, as well as demonstrated quantitative functionalization at both the C and N termini. SDS PAGE gel shift assays indicate that both termini were modified successfully, and that the introduced azides were accessible for reaction by strain-promoted azide-alkyne cycloaddition reaction. Moreover, we have obtained sufficiently high non-optimized bacterial expression yields for these modified proteins (~10 mg pure protein/L culture). Gels formed with initial moduli (G') of ~1 kPa. The system behaved as expected, demonstrating light-induced gel softening corresponding to ~15% of the initial moduli (Figure 1). Materials are cytocompatible and permit cell encapsulation.

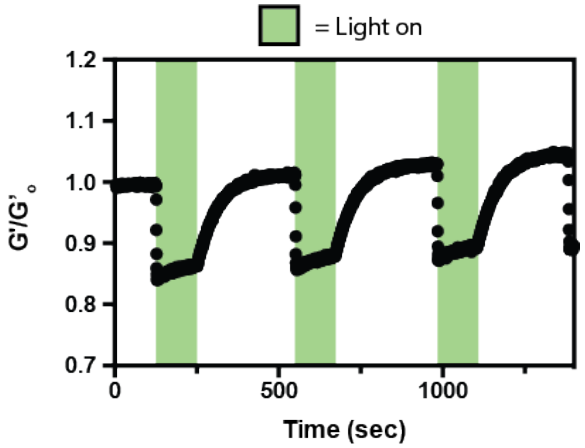

Figure 1. Photoresponsive protein-polymer hydrogels exhibit on-demand and fully reversible moduli changes in response to visible light exposure.

Conclusions: This work represents one of the first examples of the generation of fully homogeneous protein samples with bioorthogonal functionalities that can be used for gel crosslinking. Moreover, the platform yields fully reversible and dynamic changes to material stiffness in response to mild light exposure. These hydrogels represent one of the first *in vitro* cell culture platforms to mimic the anisotropic and periodic reversible stiffening that occurs *in vivo*.

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