Engineering Dynamic Reversible PEG Hydrogels using Light-sensing Proteins

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Statement of Purpose: The native cell microenvironment is extraordinarily dynamic, with reciprocal regulation pathways between cells and the extracellular matrix (ECM) guiding many physiological processes, such as cell migration, stem cell differentiation, and tissue formation. The complex interplay between spatiotemporally varying cues and cellular responses has thus far been difficult to recapitulate using *in vitro* cell culture methods. Recently, there has been an effort to introduce stimuli-responsive reversible chemistries into hydrogel-based cell culture platforms to better simulate the dynamism of the native cell environment. However, these efforts have been hampered by reliance upon chemical reactions which are irreversible, or stimulus mechanisms which are not spatially controllable or are not gentile on cells. In this study we demonstrate the use of light-sensing optogenetic proteins as a reversible, light-regulated association chemistry in the context of a biomaterial for cell culture.

Light-sensing proteins used for optogenetic research function by changing conformation in response to absorbed light. This light-mediated structural change in the protein can be engineered to control a binding or dissociation event with a binding partner. Importantly, the protein's light-triggered conformational change can be reversed, either by using a secondary wavelength of light or by waiting for the protein to undergo thermal reversion, depending on the protein. Many light-sensing optogenetic proteins can transition between their light-activated state and their dark state repeatedly with no degradation of protein function, resulting in molecular binding events that can be induced, then disrupted, reversibly using light.

In this study, we used the recently developed LOVTRAP system, consisting of the well-characterized blue light-sensing LOV2 domain and a z-affibody dubbed Zdk, to control cell adhesion to a PEG-diacrylate (PEG-DA) hydrogel. LOV2 and Zdk bind tightly in the dark with an affinity similar to that of an antibody interaction (Kd = 26.2 nM), unbind in response to blue light irradiation, and then re-bind after LOV2 undergoes thermal reversion to its dark state. This light-mediated association was used to control cell attachment to a PEG-DA gel surface by covalently immobilizing LOV2 within the gel and then displaying the adhesive peptide RGDS as a Zdk fusion on the gel surface via LOV2/Zdk interaction. Fibroblast were able to attach and spread to LOVTRAP-presented RGDS, and were subsequently lifted from the surface of the gel upon blue light exposure.

Methods: LOV2 and Zdk were expressed in E. coli and purified using Ni-NTA affinity chromatography. Zdk was expressed as a C-terminal fusion, with RGDS as an N-terminal tag, followed by an mCherry domain for ease of tracking. LOV2 was expressed with an N-terminal polyglycine sequence for site-specific PEG conjugation using sortase A, followed by an mTFP1 domain for protein stabilization and concentration measurements. PEG-DA gels with covalently immobilized acrylate-PEG-LOV2 were cast using a 380μm spacer via photopolymerization. RGDS-mCherry-Zdk or RDGS-mCherry-Zdk (non-specific adhesion control) was topically applied to the gels and allowed to bind LOV2 before rinsing the gels overnight. Fibroblasts (NIH 3T3s) were seeded onto gels and allowed to spread overnight. Gels were then imaged before and after blue light irradiation (458nm, 17.5mW/cm^2, 30 sec), and cell attachment and spreading was assessed.

Results: mCherry-Zdk was shown to preferentially associate to PEG-DA gels containing covalently immobilized LOV2. This association withstood days of rinsing, but was disrupted by blue light exposure. mCherry-Zdk release was shown to be dependent on light power, and could be spatially constrained using patterning techniques. Cells seeded on PEG-DA gels containing LOVTRAP-displayed RGDS were able to attach and spread, and then were released from the gel surface upon blue light exposure.

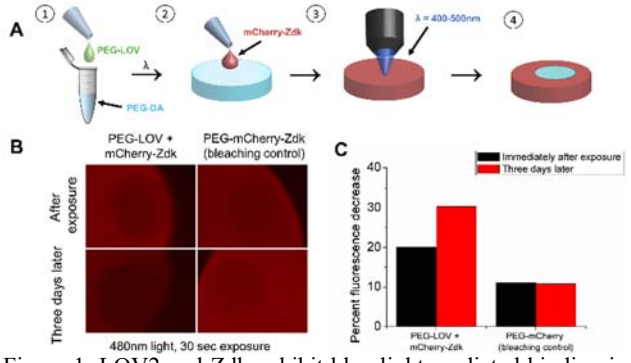

Figure 1. LOV2 and Zdk exhibit blue light-mediated binding in the context of a PEG-DA hydrogel. Zdk is tracked using mCherry fluorescence. After blue light exposure, Zdk continues to diffuse away from the exposed region, demonstrating the mCherry signal reduction is due to release and not bleaching.

Conclusions: Optogenetic proteins have a unique potential for dynamically controlling hydrogel bioactivity using light. The LOVTRAP system was able to mediate the display of a fundamental adhesion ligand on a PEG-DA surface, and in doing so granted the ability to control cell attachment on and release from the gel surface using mild blue light as a stimulus. This mechanism for dynamically presenting bioactive ligands could feasibly be used to present any biomolecule that could be chemically conjugated or recombinantly fused to Zdk in a spatiotemporally constrained way, potentially offering a far greater degree of control over transient, spatially patterned cell-instructive cues within bioactive hydrogel matrices.

References: Schultz GS. Wound Repair Regen. 2011;19;134-48, Barthes H. Biomed Res Int. 2014;2014;921905, DeForest CA. Angew Chem Int Ed Engl. 2012;51;1816-9, Miyata T. Nature 1999;399;766-9, Wang H. Nat Methods. 2016;9;755-8