Photoreversible Protein Patterning to Guide Dynamic 3D Cell Fate

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Statement of Purpose: Polymer-based hydrogels have emerged as a unique class of biomaterials that enable stem cells to be cultured in three-dimensions within near-physiological, synthetic microenvironments[1]. Recent strategies have been developed that permit bioepitopes (*e.g.*, peptides, full-length proteins) to be introduced at any point in time and space to affect cell function spatiotemporally within user-defined subvolumes of the bulk material[2-4]. While these techniques have been successfully utilized to direct a variety of basic cellular functions, advanced platforms that permit biological cues to be both *introduced* and subsequently *removed* would be beneficial in recapitulating the dynamic abundance of signaling biomolecules in the native, temporally-variable niche and in modulating complex cellular behavior[5,6]. In this work, we demonstrate that the combination of two bioorthogonal light-based chemistries provides for the reversible immobilization of full-length protein cues spatially within a hydrogel. Results further highlight the versatility of such dynamic biochemical signal presentation in better understanding basic cell physiology.

Methods: A four-arm poly(ethylene glycol) tetra(cyclooctyne) was reacted with a bis(azide), matrix metalloproteinase-degradable polypeptide as well as a photocaged alkyoxyamine in the presence of a cell suspension to form a cell-laden hydrogel network. Upon gel formation, aldehyde-functionalized proteins were patterned within the material *via* a photomediated oxime ligation using conventional photolithography (λ = 365 nm, **Figure 1**). At a later time point, the pre-patterned gel was selectively exposed to UV light, resulting in user-controlled photorelease of the patterned proteins. The kinetics of protein immobilization and subsequent release were quantified and compared to predicted values based on photokinetics as determined by NMR.

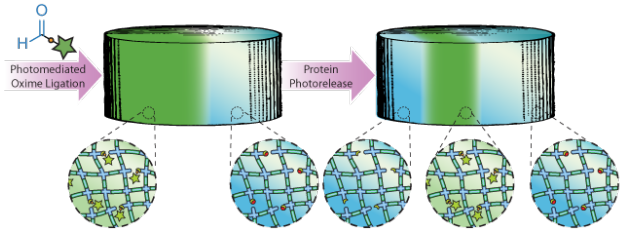

Figure 1. Post-gelation, network photocoupling and photorelease of full-length proteins

(= PEG tetracyclooctyne, = peptide bis(azide), = alkoxyamine, = photocage, = protein of interest, = photocleavable linker with aldehyde)

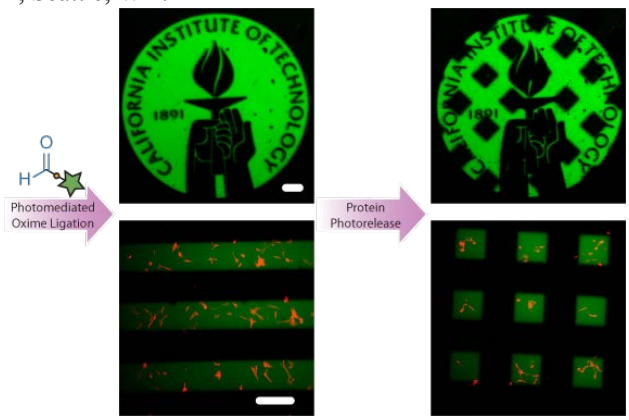

Figure 2. Fluorescent proteins are stereolithographically photopatterned into the gel and subsequently removed with full spatiotemporal control (top) to regulate dynamic changes in 2D cell attachment (bottom) (scale bars = 200 μm)

Results: High cell viability was observed for initial gel formation, photocoupling reactions, as well as photorelease. Full-length proteins were successfully patterned with micron-scale resolution **(Figure 2)**. Upon exposure to UV light, the protein was successfully patterned in and then released in a predictable fashion. The entire patterning process could be completed in just a few hours, thereby defining the time scale over which biological functions can realistically be controlled within this platform. By selectively photocoupling vitronectin (65 kDa) into a cell-laden hydrogel network and subsequently removing it, user-directed morphological, migratory, and differentiation changes were induced within, and confined to, the patterned regions for hMSCs.

Conclusions: This work represents a synthetic approach that enables the direct fabrication of gels with ideal network structures that can be independently (de)functionalized and all in the presence of cells. A material that affords this level of spatial and biomolecular control should provide a useful tool for 3D cell culture to answer complex biological questions and to regenerate fully-functional tissue *ex vivo*.

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