Photopatterned Immobilization of Site-Specifically Modified Proteins within 3D Gels

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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]. Recently developed strategies permit user-defined spatiotemporal introduction of bioepitopes to control cell function within subvolumes of the bulk material[2-4]. Though such initial attempts have proven successful in the tethering of small molecules and short synthetic peptides within 3D culture platforms, there is a growing interest to direct cell fate through the patterned immobilization of *full-length proteins*[5,6]. The high degree of protein-substrate specificity, as well as their ability to modulate complex cellular behavior (*e.g.*, stem cell differentiation, protein secretion, and cell-cell interactions) generally exceeds that from simple chemical moieties. In this work, we demonstrate that site-specifically-modified proteins can be immobilized within a 3D material using a bioorthogonal light-based chemistry. As the exact residue of protein conjugation to the material can be explicitly defined *a priori*, proteins maintain wild-type levels of bioactivity and substrate specificity throughout modification and usage. Results further highlight the versatility of such dynamic biochemical signal presentation to better understand basic cell physiology.

Methods: Chemoenzymatic strategies were used to introduce reactive aldehydes at user-defined sites on recombinant proteins of interest. Cell-laden hydrogel networks were formed upon reaction of a four-arm poly(ethylene glycol) tetra(cyclooctyne), a bis(azide), matrix metalloproteinase-degradable polypeptide, as well as a photocaged alkoxyamine in the presence of a cell suspension. Upon gel formation, aldehyde-functionalized proteins were patterned within the material *via* a photomediated oxime ligation using conventional photolithography (λ = 365 nm, **Figure 1**). The kinetics of protein immobilization were quantified and compared to predicted values based on photokinetics as determined by NMR.

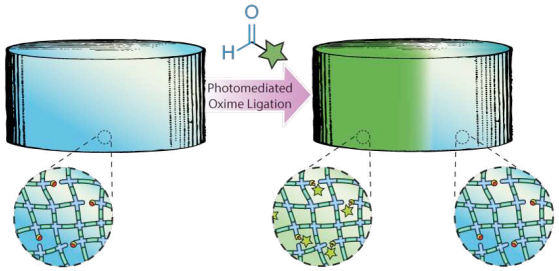

Figure 1. Post-gelation, network photocoupling of site-specifically-modified full-length proteins
(= PEG tetracyclooctyne, = peptide bis(azide), = alkoxyamine, = photocage, = protein of interest)

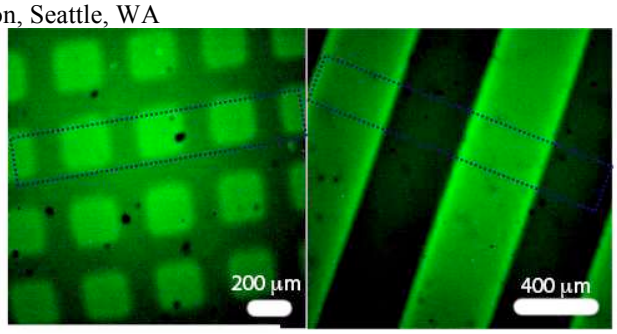

Figure 2. Fluorescent proteins are photolithographically patterned into the gel to ultimately regulate changes in 3D cell function.

Results: Site-specifically modified proteins containing a single aldehyde were expressed recombinantly and purified with good yield (~20 mg/L culture). High cell viability was observed for initial gel formation and photocoupling reactions. Full-length proteins were successfully patterned with micron-scale resolution **(Figure 2)**. The 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 aldehyde-functionalized vitronectin (65 kDa) into a cell-laden hydrogel network, 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 functionalized with site-specifically modified proteins 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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