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. Recently developed strategies permit user-defined spatiotemporal introduction of bioepitopes to control cell function within subvolumes of the bulk material. 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. 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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References: