Photoreversible Patterning of Biomolecules within 3D Click Gels

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Statement of Purpose: "Click chemistry" represents an emerging paradigm in organic synthesis and involves highly selective and orthogonal reactions that proceed rapidly and under a variety of mild conditions[1]. This work illustrates a synthetic strategy where cytocompatible, regularly-structured hydrogels form rapidly *via* a copper-free click chemistry[2,3]. Incorporated into the hydrogel backbone is a photoreactive C=C ene functionality, which enables any thiol-containing molecule (including cysteine-containing peptides) to be pendently *added* at any point in time and space within the gel *via* the thiol-ene click reaction. By photopatterning biomolecules that contain a photodegradable nitrobenzyl ether linker, these peptides can be subsequently *removed* with the same level of spatiotemporal control. These unique attributes enable 3D cell behavior to be assayed within specific user-defined regions of the material based on both the introduction of a chemical cue as well as its removal.

Methods: A four-arm poly(ethylene glycol) tetra(difluorinated cyclooctyne) was reacted with a bis(azide), allyl ester-containing polypeptide in the presence of a cell suspension to form a cell-laden hydrogel network. Upon gel formation, fluorescently-labeled peptides were pendently photocoupled within the material using conventional visible light (435 nm) photolithography (**Figure 1**). At a later timepoint, the gel was selectively exposed to UV light (365 nm), resulting in user-controlled photorelease of the patterned peptides. The kinetics of peptide release were quantified and compared to predicted values based on photodegradation kinetics as determined by NMR.

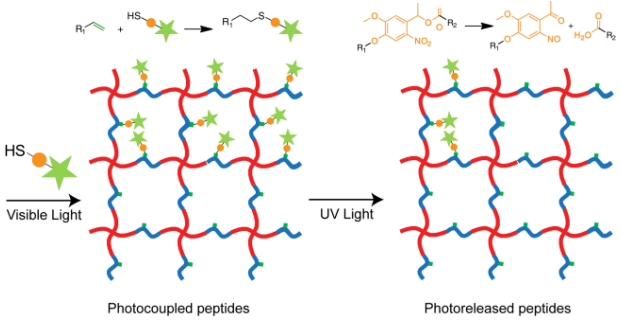

Figure 1. Post-gelation, network photocoupling and photorelease of biomolecules

(= PEG tetracyclooctyne, = peptide bis(azide), = photodegradable linker, = photopatternable group, = biomolecule of interest)

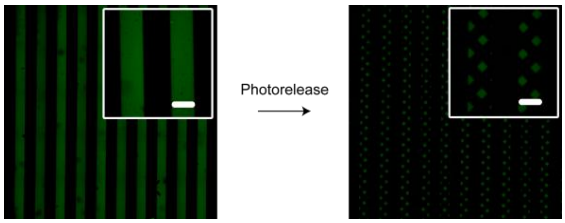

Figure 2. Fluorescent peptides are stereolithographically photopatterned into the gel and subsequently removed with full spatiotemporal control (scale bar = 200 μm)

Results: High cell viability (>95%) was observed for initial gel formation, photocoupling reactions, as well as photorelease. Small molecules and peptides were successfully patterned with micron-scale resolution **(Figure 2)**. Upon exposure to UV light, the peptide was successfully released in a predictable fashion (**Figure 3**). Photofunctionalization was found to occur orthogonally to photodegradation over the chosen wavelengths, signifying independent control over the two photoreactions. By selectively photocoupling the fibronectin-derived RGDS motif to a cell-laden hydrogel network and subsequently removing it, user-directed morphological and migratory changes were induced within, and confined to, the patterned regions for NIH 3T3s and hMSCs.

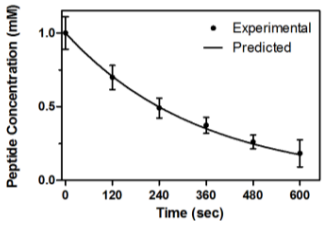

Figure 3. The amount of released peptide is predicted for a given light condition and exposure time based on independently-determined photodegradation kinetics

Conclusions: This work represents a synthetic approach that enables the direct fabrication of gels with ideal network structures that can be independently functionalized and defunctionalized and all in the presence of cells. A material that affords this level of spatial and biomolecular control will become increasingly important in probing more complex biological questions and attempting to recreate fully-functional tissue *ex vivo*.

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