Phototunable Click-based Hydrogels for 3D Cell Encapsulation and Manipulation

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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 are photodegradable linkers[4] and photoreactive functionalities, whose reaction can be independently controlled using different wavelengths of light. When exposed to visible light, the thiol-ene click reaction enables any thiol-containing molecule (including cysteine-containing peptides) to be pendently *added* within the gel. Alternatively, when the gel is exposed to UV light, photomediated crosslink *removal* occurs and enables user-defined mass loss and erosion. The local manipulations of physical and chemical properties of the gel microenvironment provide an avenue to detect and/or direct cell function throughout specific regions within the 3D material.

Methods: A four-arm poly(ethylene glycol) tetraphotolabile-azide was reacted with a bis(difluorinated cyclooctyne), allyl ester-containing, MMP-cleavable polypeptide[5] in the presence of a cell suspension to form a cell-laden hydrogel network (**Figure 1**). The kinetics of this step-wise polymerization was characterized with rheometry and MAS-NMR. Upon formation of the initial gel, fluorescently-labeled peptides were pendently photocoupled within the material using conventional visible light (435 nm) photolithography. In addition, the gel was selectively exposed to UV light (365 nm), resulting in user-controlled photodegradation.

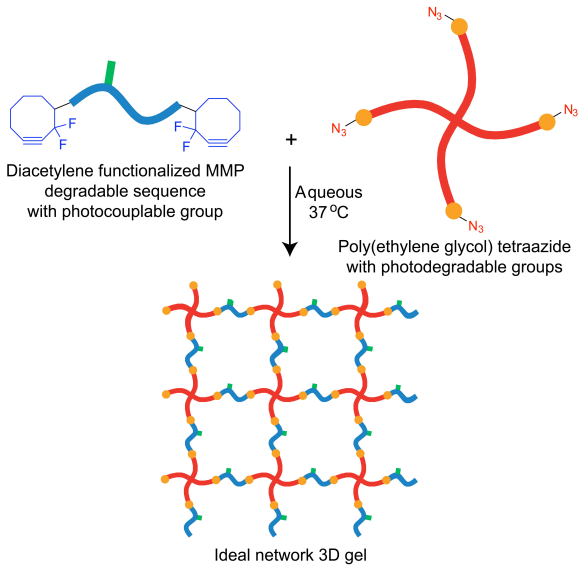

Figure 1. Ideal network hydrogel formation scheme (● = photodegradable linker, ▮ = photopatternable group)

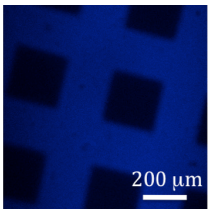

Figure 2. Fluorescent peptides (blue) are stereolithographically photopatterned into the gel at defined locations and imaged with confocal microscopy

Results: High cell viability (>95%) was observed for initial gel formation, photocoupling reactions, as well as photodegradation. Small molecules and peptides were successfully patterned within the material spanning intricate three-dimensional structures with micron-scale resolution **(Figure 2)**. Complex voids were etched within the hydrogel network in user-specified patterns (**Figure 3**). Photofunctionalization was found to occur orthogonally to photodegradation over the chosen wavelengths, signifying independent tunability of the system's chemical and physical properties. By selectively photocoupling the fibronectin-derived RGDS motif to a cell-laden hydrogel network and degrading specific channels for migration, user-directed morphological and migratory changes were induced within, and confined to, the patterned regions for NIH 3T3s and hMSCs.

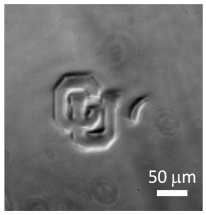

Figure 3. Distinct voids created within hydrogel network *via* multiphoton photodegradation imaged in brightfield

Conclusions: This work represents a synthetic approach that enables the direct fabrication of biologically functionalized gels with ideal structures that can be independently photofunctionalized and photodegraded 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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