# Sequential Click Reactions for Synthesizing and Patterning 3D Cell Microenvironments

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**Statement of Purpose:** "Click chemistry" promises extremely selective and orthogonal reactions that proceed with high efficiency under a variety of mild conditions[1,2]. These independently modular reactions enable the facile synthesis of functional molecules, and ultimately materials with highly defined properties. While the versatility of click reactions has been broadly exploited, a major limitation is the intrinsic toxicity of the synthetic schemes and the inability to translate these approaches to biological applications. This work illustrates a synthetic strategy where macromolecular precursors react via a copper-free click chemistry[3], allowing for the direct encapsulation of cells within click hydrogels for the first time. Subsequently, an orthogonal thiol-ene photocoupling chemistry is introduced that enables patterning of biological functionalities within the gel, allowing one to tailor the physical and chemical properties of the cell culture niche *in situ*. These local manipulations of the gel microenvironment provide an avenue to introduce chemical cues that direct cell function and/or assay cell behavior throughout specific regions within the material.

**Methods:** A four-arm poly(ethylene glycol) tetraazide was reacted with a bis(difluorinated cyclooctyne), allyl ester-containing, MMP-cleavable polypeptide[4] in the presence of a cell suspension to form a cell-laden hydrogel network (**Figures 1 & 2**). The kinetics of gelation of this step-wise polymerization was fully characterized using MAS-NMR and rheometry, each of which indicates an ideal network structure, with the latter also providing a real-time measurement of the evolving hydrogel bulk material properties. Upon formation of the initial gel, fluorescently-labeled peptides were pendently patterned throughout the material using conventional photolithography, as well as two-photon techniques.

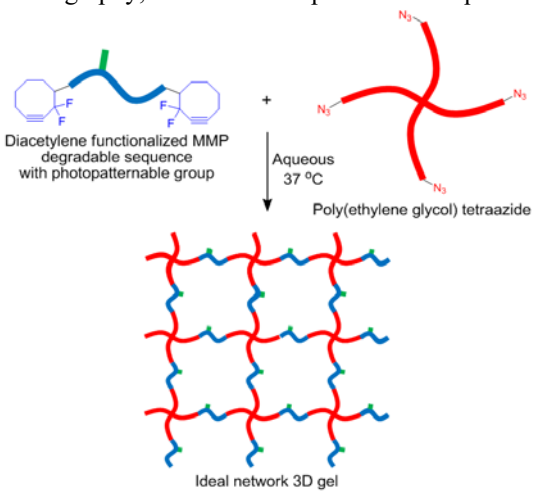


Figure 1. Ideal network hydrogel formation scheme

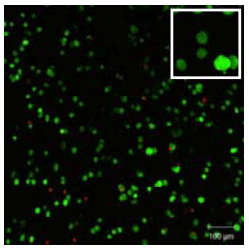

Figure 2. Live/dead confocal image of cells 24 hours after encapsulation (scale bar = 100 μm)

**Results:** High NIH-3T3 cell viability (>95%) was observed for both the initial gel formation reaction (which occurred within 5 minutes of monomer combination) as well as in subsequent patterning reactions (**Figure 2**). Small molecules and peptides were successfully patterned within the material in complex three-dimensional structures with micron-scale resolution (**Figure 3**). More specifically, a cysteine-containing fibronectin RGD motif was selectively photocoupled to a cell-containing hydrogel network and observed to locally induce morphological and migratory changes within the functionalized regions. These induced differences in cell behavior can be confined to patterned regions within a single gel.

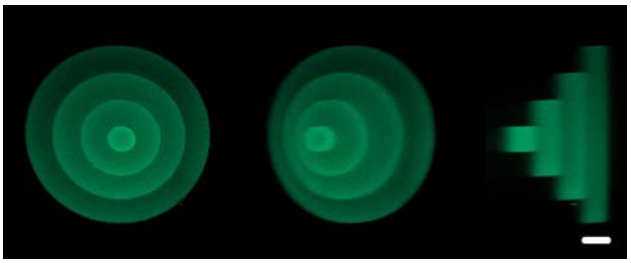

Figure 3. Two-photon patterning of fluorescently-labeled peptides within hydrogel network (scale bar = 50 μm)

**Conclusions**: This work represents a synthetic approach that allows for the direct fabrication of biologically functionalized gels with ideal structures that can be photopatterned 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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**References:**

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