Hybrid Photopatterned Enzymatic Reaction (HyPER) for in Situ Cell Manipulation

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Statement of Purpose: The ability to design artificial extracellular matrices as cell instructive scaffolds has opened the door to technologies capable of studying cell fate in vitro and to guide tissue repair in vivo. One main component of the design of artificial extracellular matrices is the incorporation of biochemical cues to guide cell phenotype and multicellular organization. The extracellular matrix is composed of a heterogeneous mixture of proteins that present a variety of spatially discrete signals to residing cell populations. In contrast, most engineered ECMs do not mimic this heterogeneity. In recent years the use of photodeprotection has been used to achieve spatial immobilization of signals. However, these approaches have been limited mostly to small peptides. Here we combine photodeprotection with enzymatic reaction to achieve spatially controlled immobilization of active bioactive signals that range from small molecules to large proteins. A peptide substrate for transglutaminase factor XIII (FXIIIa) is caged with a photodeprotectable group, which is then immobilized to the bulk of a cell compatible hydrogel. With the use of focused light the substrate can be deprotected and used to immobilize patterned bioactive signals. This approach offers an innovative strategy to immobilize delicate bioactive signals, such as growth factors, without loss of activity and enables In situ cell manipulation of encapsulated cells.

Methods: Our platform uses activated transglutaminase factor XIII (FXIIIa) catalyzed reaction to immobilize signals to light-activated regions of the hydrogel. To control enzymatic activity we employ a caged enzyme substrate (K-peptide) that is immobilized to the backbone of the hydrogel matrix. The cage prevents enzymatic attachment of the signal before light exposure. Following localized removal of the light-sensitive cage, FXIIIa catalyses the formation of a stable amide bond between the desired bioactive signal which contains a second substrate for FXIIIa (Q-peptide).

Results: Caged K-peptide substrate synthesis: The o-NB molecule was synthesized from vanillin and reacted directly with the full length peptide. Caged K-peptide photo-deprotection kinetics and FXIIIa immobilization can be predicted: Using controlled deprotection we determined the amount of immobilized Q-RGD bioactive signal can be predicted. HyPER can achieve spatial co-immobilization: immobilization of different bioactive signals was achieved with 2D and 3D control (fig. 1). Using HyPER for in situ cell manipulation: HyPER was performed in the presence of cells to show safety, toxicity and effect. MSCs responded to the pattern both in 2D and 3D. In 2D cells only spread in RGD areas and in 3D MSCs showed different cell morphology when exposed to PDGF gradients than when a homogeneous immobilization strategy was used.

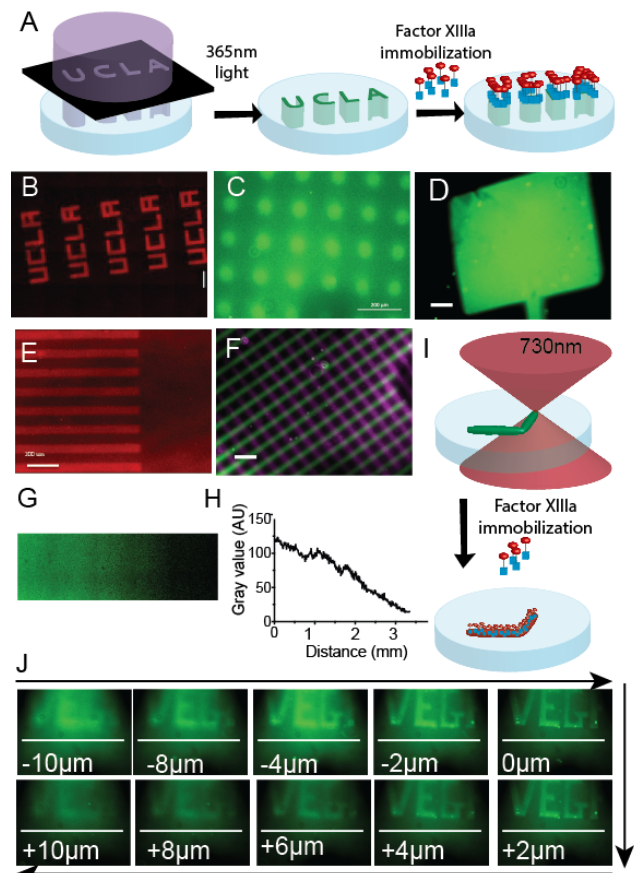

Figure 1. HyPER patterning and immobilization strategy (A). Immobilization of small molecules (B) RGD peptides (C,D), fibronectin fragments (E), co-patterning of RGD and PDGF (F) RGD in a linear gradient (G,H) and two photon immobilization of VEGF (I, J).

Conclusions: In summation, we have developed a high yield method for direct caging of a FXIIIa-recognized substrate, K-peptide. Using light we were able to deprotect the FXIIIa substrate and then use FXIIIa to catalyze the immobilization of bioactive signals. Our studies have shown the resultant platform displays quantifiable uncaging behavior, resulting in predictable immobilization behavior. The enzymatic immobilization chemistry is highly modular, allowing for spatially-defined patterns of a wide variety of active signals, including oligopeptides, protein fragments, and growth factors. We demonstrated patterning in 2D using photomasks and optical filters to produce both binary and gradient patterns, respectively, and in 3D via a previously developed multiphoton uncaging technique. Finally, we demonstrated the capacity of our patterning platform to control cell behavior through the alteration of cell morphology, viability, and proliferation. In addition, we demonstrate the ability to modify the microenvironment of cells in situ without cell damage.

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