

Micropatterned hydrogel layers for Tissue Engineering

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INTRODUCTION: One of the main challenges in the field of Tissue Engineering is the construction of functional and well organized 3-D tissues. For this purpose, distribution of cells, as well as biological cues involved in tissue formation, must be tightly controlled.

We propose to pattern synthetic hydrogels with cell-instructive motifs, such as growth factors (e.g.: VEGF) and peptides sequences known to activate specific signaling pathways (e.g.: RGD), in order to control cell differentiation and function into the desired cell phenotype. First, we want to create patterned surfaces in order to study cell behaviour in a simplify 2-D model.

To create such cell instructive surfaces, fibrin-like synthetic Poly(ethylene glycol) hydrogels^{1,2}, which allow simultaneous incorporation of biomolecules during gel formation, are used. The hydrogel cross-linking mechanism is based on a transglutaminase, called Factor XIII, that catalyzes an acyl-transfer reaction between two substrates: TG and Lys (*Fig.1*), resulting in an isopeptide bridge. In a first approach, we pattern on a non-fouling PLL-g-PEG background the TG domain, which has been previously grafted to PLL-g-PEG (PLL-g-PEG-TG), by Molecular Assembly Patterning by Lift-off (MAPL)³. This technique has been successfully used to pattern RGD³ on a non-fouling background. The TG domain at the end of the PLL-g-PEG could be used as a liker to selectively bind Lys-modified molecules (e.g.: 8 arm Lys-PEG), which could be further used to bind TG-modified molecules (e.g.: TG-VEGF).

METHODS: First, PLL-g-PEG-TG (*Fig.1*) was immobilized on a Nb₂O₅ wafer (60µm x 60µm) previously patterned with a photoresist. After removal of the photoresist, PLL-g-PEG was used as back fill. In a second step, 8-arm Lys-PEG was bind in presence of FXIIIa (10 U/ml) and Ca²⁺ (50 mM) to the TG domain tether to PLL-g-PEG. Afterwards, TG-VEGF was linked to 8-arm Lys-PEG. Presence of VEGF was detected with a first anti-VEGF antibody and a FITC-labelled secondary antibody (*Fig.2*).

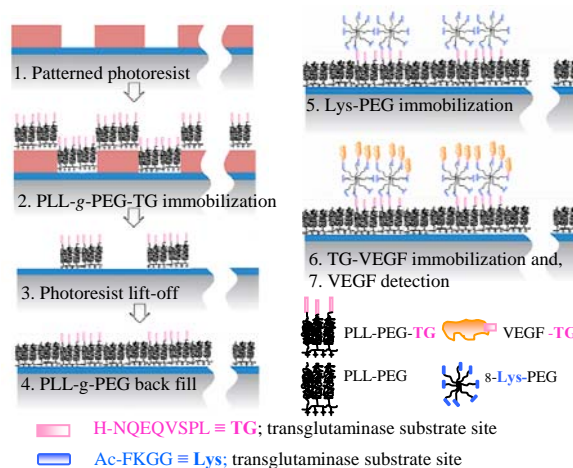


Fig. 1: Schematic figure of the patterning approach.

RESULTS & DISCUSSION: In *Fig. 2* we can see the expected pattern. In green, TG-VEGF is detected in areas where PLL-g-PEG-TG was immobilized. The black pattern corresponds to areas where PLL-g-PEG was used as back fill. However, further studies are needed to demonstrate; 1) a pattern of PLL-g-PEG-TG on a PLL-g-PEG background, 2) selective linkage of Lys-PEG to the TG domain on the PLL-g-PEG-TG molecule, and 3) covalent linkage of TG-VEGF to 8-arm Lys-PEG.

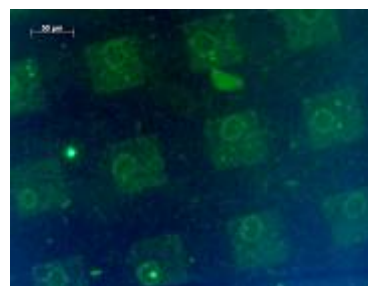


Fig. 2: TG-VEGF pattern on 60µm x 60µm Nb₂O₅ wafer.

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