Three Dimensional Photolithographic Patterning of Proteins in Hydrogels for Fabrication of Gradient Biomaterials to Direct Cell and Vessel Growth

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Statement of Purpose: Spatial control over vessel formation is critical as undirected vessel growth can lead to vessel instability and poor network functionality. Two photon laser scanning lithography (TP-LSL) can be used to control the localization of peptides and proteins, like angiogenic growth factors, within synthetic materials [1]. In this method, the simultaneous absorption of two photons from a high frequency pulsed laser allows for excitation of an initiator molecule to be limited to a micro-scale focal volume, locally triggering photo-reactive processes with microscale 3D control. In this work, vascular endothelial growth factor (VEGF) was patterned into proteolytically degradable, cell adhesive poly(ethylene glycol) diacrylate-based hydrogels. By controlling both the precise location and concentration of VEGF within a 3D hydrogel, we were able to pattern protein gradients that mimic the biochemical signaling found in the tissue microenvironment and were able to spatially guide vessel formation.

Methods: PEG-GGGPQGIWGQGK-PEG (PEG-PQ) and PEG-RGDS tagged with Alexa Fluor 647(AF647) was synthesized as previously described [1]. For other studies, PEG-VEGF was labeled with AF647. The PQ peptide sequence is MMP2 sensitive and was incorporated into the polymer backbone to render the hydrogels biodegradable. 5% (w/v) PEG-PQ hydrogels were photopolymerized under white light using eosin Y as a photoinitiator. Hydrogels were soaked overnight in 2 µM PEG-VEGF-AF647 with sterilize1.5%(v/v) triethanolamine,1mM eosinY, and 3.95µL/mL N-vinyl pyrrolidone in 10 mM HBS. Design of 3D patterns of PEG-VEGF was specified using the region of interest (ROI) function in Olympus software. For patterning experiments, a 25X objective with NA of 1.05 was used and the Ti:Saph fsec pulsed laser on the Olympus FV100 multiphoton system was set to an excitation wavelength of 720 nm. An array of 21 distinct square patterns of fluorescent VEGF within a PEG-DA hydrogel was patterned. Each square was fabricated via the selection of a unique combination of laser intensity ranging from 4 of 38 mW and scan speed ranging from 8 to 40 µs per pixel. Amount of protein was quantified by measuring the fluorescence from each box and compared against a standard curve of PEG gels with known concentrations of PEG-VEGF-AF647. A linear concentration gradient of covalently immobilized PEG-VEGF-AF647 was created by controlling the scanning number and ROIs. To study the formation of vascular structures within the gradient, human umbilical vein endothelial cells (HUVEC) and human vascular pericytes (HVP) at 30,000 cells/µL at a ratio of 4:1 of HUVEC to HVP were suspended in 5% PEG-PQ. A 5 µL droplet was then placed adjacent to a hydrogel containing a PEG-VEGF gradient and photopolymerized to create a singular gel. Hydrogels were immersed in EGM-2 media without VEGF and cultured for 10 days. Tubulogenesis of the encapsulated cells and invasion into the hydrogel gradient was assessed by immunostaining ECs with CD31 and pericytes with α-smooth muscle actin (αSMA).

Results: 21 distinct square patterns of PEG-VEGF-AF647 within a PEG-PQ hydrogel are shown in Figure 1. A protein concentration profile of this pattern shows that longer laser dwell times and higher laser power correlated with higher immobilization of fluorescent VEGF molecules. The concentration and location of PEG-VEGF immobilized 3D within the hydrogel can be controlled. By utilizing this technique, a VEGF gradient with a known slope of 1.34 $ngmL^{-1}\mu m^{-1}$ was immobilized in a degradable PEG hydrogel. Length of endothelial tubule invasion up the gradient was evaluated and compared to hydrogels with no VEGF and homogeneous VEGF (Figure 2). We demonstrate that ECs can be guided to form tubule-like structures in response to a gradient of immobilized VEGF. Alignment of tubule structures was observed in the direction parallel to the gradient. Furthermore, the length of tubule invasion was found to be significantly longer ($p<0.05$) in the direction parallel to protein gradient as compared to control hydrogels with no VEGF and VEGF immobilized homogenously in bulk.

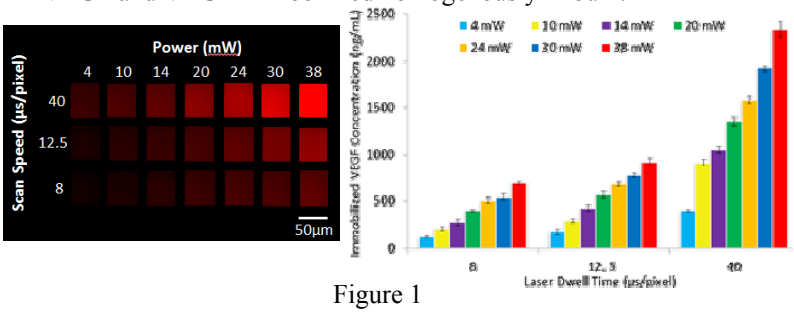

Figure 1

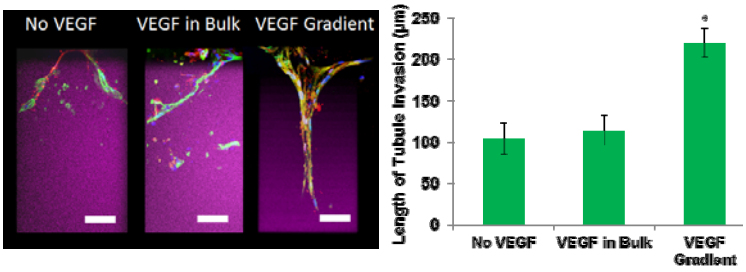

Figure 2

Conclusions: This study offers insight in design of synthetic scaffolds that can be used to promote directed and guided vessel formation within engineered tissues. By utilizing TP-LSL, we demonstrate control over spatial localization of protein signaling and create biomolecule gradients that can be tuned and modulated for mimicking physiological signaling in 3D PEG hydrogels. In scaffolds containing these gradients, tubule sprout invasion was observed to be oriented in the direction of the gradient with tubule invasion length being significantly greater than the control hydrogels.

Reference: [1] Hoffmann JC, West JL. Soft Matter. 2010. 6: 5056--63.