Patterned Surface Immobilization of Vascular Endothelial Growth Factor to Promote Endothelial Cell Tubulogenesis

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Statement of Purpose: A major limitation for tissue engineered products is the lack of microvascularization required for nutrient, oxygen, and waste transport. Poly(ethylene glycol) (PEG) is a biocompatible, hydrophilic polymer which can be crosslinked into hydrogels with tunable mechanical properties, suitable for use as a tissue engineering matrix for soft tissues. PEG hydrophilicity prevents protein adsorption and subsequent cell adhesion unless modified with cell adhesive substrates[1]. PEG can also be modified with covalently-bound growth factors to influence cell behavior[2]. Through photolithographic patterning, we have been able to control the covalent attachment of cell adhesive peptides and vascular endothelial growth factor (VEGF) to the hydrogel surface in an effort to create the high organization of complex tissues and microvasculature essential to tissue function.

Methods: PEG-diacrylate (PEG-DA) was prepared by combining dry PEG (MW 6000Da, Fluka), acryloyl chloride, and triethylamine in anhydrous dichloromethane (DCM). The resulting solution was washed with K_2CO_3 and separated into aqueous and DCM phases to remove HCl. The DCM phase was dried with anhydrous $MgSO_4$. PEGDA was precipitated, filtered, and dried under vacuum. PEG-DA hydrogels were formed using 6KDa PEG-DA dissolved in HBS, a photoinitiator, and white light. Cell-adhesive peptide RGDS and endothelial-specific angiogenic growth factor rhVEGF-165 (Peprotech) were each conjugated to 3.4KDa Acryl-PEG-NHS (Nektar). Conjugation was confirmed via SDS-PAGE with silver staining.

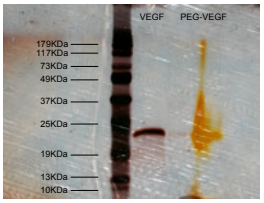

Figure 1. SDS-PAGE gel showing PEG conjugation to VEGF indicated by the increase in MW corresponding to the attachment of PEG chains[2].

Acryl-PEG-RGDS was conjugated to a fluorescent tag by combining Acryl-PEG-RGDS and AlexaFluor 488 (Molecular Probes) in a 1:10 molar ratio in 50mM sodium bicarbonate buffer at pH 8.5. Acryl-PEG-RGDS and Acryl-PEG-VEGF were crosslinked to the surface of PEG-DA hydrogels at concentrations of 30E-6mol/ml PEG-RGDS and 4.2E-10mol/ml PEG-VEGF using a photoinitiator and laser. On "unpatterned" gels, RGDS and VEGF surface modification covered the entire surface of the gel. A pattern of a 10µm-width line was etched onto a chrome photomask by a DWL66 mask maker

(Heidelberg Instr). On "patterned" gels, surface modification with RGDS and VEGF was restricted to specific regions, using laser light shining through the chrome photomask. Human umbilical vein endothelial cells (HUVECs) (Cambrex) were seeded onto the surface of the hydrogels at 8.4E4 cells/cm^2.

Results/Discussion: After 20 days, HUVECs formed tube-like structures on unpatterned gels presenting RGDS and VEGF on the surface, but significantly fewer tube-like structures on gels presenting only RGDS.

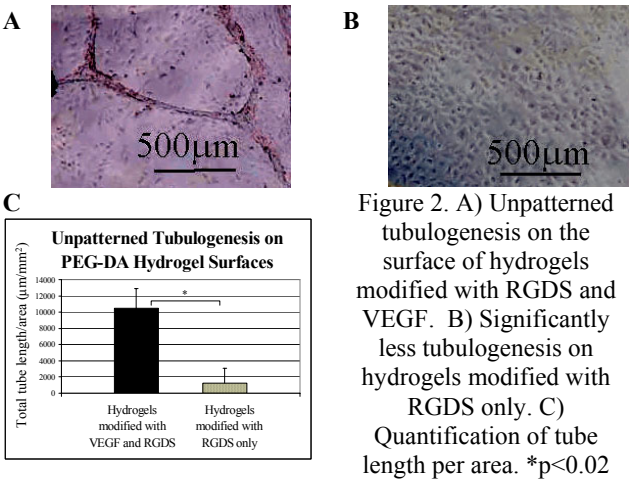

Figure 2. A) Unpatterned tubulogenesis on the surface of hydrogels modified with RGDS and VEGF. B) Significantly less tubulogenesis on hydrogels modified with RGDS only. C) Quantification of tube length per area. *p<0.02

HUVECs on patterned gels modified with RGDS and VEGF attached exclusively to the areas patterned (~10µm width lines) and appear to form tube-like structures within two days.

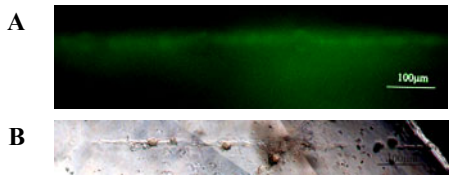

Figure 3. A) Patterned PEG-RGDS-Fluor and PEG-VEGF 10µm line. B) HUVECs attached to same line.

Conclusions: This research leads to future work entailing the co-culture of tissue-specific cells and capillary cells constrained by patterning of cell-specific adhesive peptides and growth factors to create intricate tissue organization and cell-cell cooperation to provide tissue function in both two and three-dimensional patterned PEG-DA scaffolds.

References:

1] Gombotz WR. Biomed Mater Res. 1991; 25:1547.
2] Delong S.. Biomaterials. 2005; 26: 3227-34.