BioArtificial Matrices to Promote Vascular Network Formation

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Statement of Purpose: The ability of engineered tissue substitutes to recapitulate normal blood vessel wiring is central to their successful integration with host tissue, proper physiological function, and long term survival. While a variety of tissue engineering strategies have shown successful blood vessel growth into implants these approaches do not recapitulate normal blood vessel wiring. In the engineering of structurally complex tissue types, the growth pattern of blood vessels is essential for correct function. We developed a strategy to direct the formation of orderly networks of blood vessels in artificial bioactive materials. We modified published protocols[1] to engineer poly(ethylene glycol) (PEG)-based hydrogels presenting patterns of cell adhesive motifs that promote endothelial cell adhesion and organization on 2D surfaces. We are currently adapting the technique to 3D systems.

Methods: Photolithography masks with varying line widths of were designed in AutoCAD and laser plotted on Mylar film. The bioadhesive peptide GGRGDSPGGK-carboxyfluorescein was conjugated to a PEG spacer arm by reacting with acrylate-PEG-SCM (MW 3400). Human plasma fibronectin (FN) was also conjugated to acrylate-PEG-SCM at free amines. PEG-based hydrogels composing of acrylate-PEG-acrylate (PEGDA, MW 3400) dissolved in PBS at 0.1g/mL were cast in silicone isolator wells and polymerized by exposure to UV light. After polymerization a thin layer of A-PEG-RGD or A-PEG-FN in PBS was pipetted onto the gel surface and overlain with a photolithography mask. A-PEG-RGD or A-PEG-FN was conjugated to the PEGDA hydrogel surface by a second UV exposure. The patterned gel was washed extensively to remove any non-tethered background ligand. Human umbilical vein endothelial cells (HUVEC) at P4 in EGM-2 media were seeded on the surface of the hydrogel at 10,000 cells/cm2, incubated at 37°C, 5% CO2, and examined at 24 hours and 4 days later. The patterned cells were fixed and stained for DNA, F-actin, and VE-cadherin to image via fluorescence microscopy.

Results: Using the photolithography system, we successfully created geometries resembling branching vasculature with sharply defined features as small as 10 µm of both fluorescein-labeled RGD peptide and AMCA-labeled FN. HUVEC seeded on the gel adhered only to the patterned ligand and aligned along the pattern forming continuous structures over several millimeters. These structures were stable for a minimum of four days in culture, when they were fixed for staining. Notably, HUVEC adhered to microcarriers seeded onto the surface of patterned gels migrated off of the microcarriers and out along the adhesive ligand pattern. After four days in culture, aligned HUVEC on the 50 µm width FN patterns began to form structures resembling extended tubules upon inspection with bright field microscopy. While cells adhered and aligned with all other pattern widths examined, the 50 µm pattern was the only one that promoted a noticeable structural change. Fluorescent microscopy revealed actin cytoskeletons outlining structures resembling lumens. Extended cell-cell junctions incorporating VE-cadherin indicate endothelial cell-cell organization.

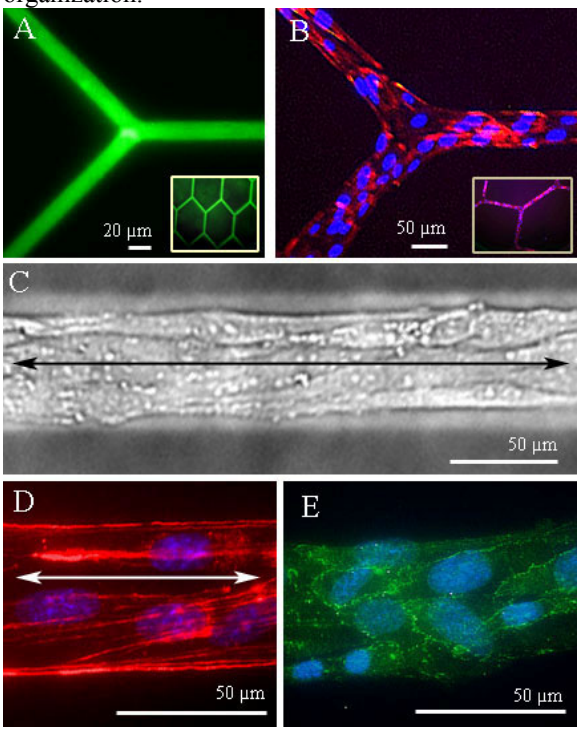

Figure 1. (A) FITC-labeled RGD pattern, inset reduced magnification. (B) HUVEC on 50 µm FN pattern (DNA = blue, F-actin = red), inset reduced magnification. (C) DIC of HUVEC aligned on FN, structure resembles capillary with lumen. (D) HUVEC on 50 µm FN pattern (DNA = blue, F-actin = red), F-actin stain outlines lumen. (E) HUVEC on 50 µm FN pattern stained for VE-cadherin (green) showing cell-cell junctions.

Conclusions: These results indicate that we can direct endothelial cell sprouting and control the formation of specific structural geometries on PEG hydrogels. Future experiments will confirm the presence of lumens in the structures. We plan to adapt this model to a 3D system as well as incorporate other bioactive molecules to create more specific and complex materials capable of growing ordered networks of vessels when implanted in vivo.

References: 1. Hahn MS, Taite LJ, Moon JJ, Rowland MC, Ruffino KA, West JL. Biomaterials, 27, 2519, 2006.

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