Endothelial tubulogenesis on surface-patterned PEG hydrogels

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Introduction

Density and geometry of ECM coated substrate can be controlled precisely with various micropatterning techniques available. Using these techniques, ECs have been previously micropatterned in stripes on gold and chitosan surfaces. [1, 2] We have developed surface patterning techniques for PEG hydrogels based on photolithography. [3] In this work, we report applications of the surface-patterned PEG hydrogels to regulate capillary morphogenesis and to investigate the progress of tubulogenesis.

Materials and Methods

PEG-diacrylate (MW 6000) was synthesized by reacting PEG with acryloyl chloride in the presence of triethylamine. The cell adhesion peptide RGDS was coupled to PEG monoacrylate by reaction with acryloyl-PEG-N-hydroxysuccinimide (MW 3400). To pattern RGDS on PEG hydrogels, the base PEG-diacrylate hydrogels were photopolymerized first. A prepolymer solution containing acryloyl-PEG-RGDS was applied on the hydrogel. A transparency mask was laid, and the gel was exposed to the UV lamp again. To visualize patterned areas on the hydrogels, FITC-conjugated PEG was patterned and examined with fluorescence microscopy. In order to quantify the amount of peptides immobilized on the hydrogels, acryloyl-PEG-RGDS was immobilized on PEG hydrogels without patterns, and the amine concentration was measured with acid digestion and ninhydrin assay. Human umbilical vein endothelial cells (HUVECs) were seeded on PEG hydrogels patterned with RGDS. At various time points, the HUVECs were fixed, stained with phalloidin-TRITC and DAPI, and visualized with confocal microscopy.

Results and discussion

The surface concentration of peptide can be controlled through concentration of acryloyl-PEG-RGDS as well as the UV irradiation time. (Fig 1)

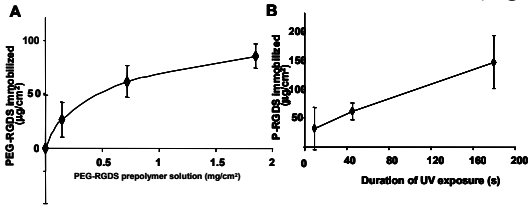

Fig 1. The amount of RGDS immobilized on the PEG hydrogels depends on A) RGDS concentration in the prepolymer solution, and B) the duration of exposure to the UV light during photopolymerization.

Using FITC-conjugated PEG, stripes with width corresponding to the original patterns were visualized, confirming successful surface patterning on the hydrogels. (Fig 2A, B) Hydrogels with patterned RGDS (300 μg/cm^2) were seeded with HUVECs, and cell adhesion and spreading were found to be restricted to the patterned stripes. (Fig 2C) HUVECs cultured for 10 days underwent morphogenesis and formed tube-like structures in the middle of stripes. (Fig 2D) After 18 days in culture, HUVECs plated on 70 μm wide stripes have formed extensive tubules reaching several mm in length (Fig 2E) whereas the cells on 200 μm wide stripes were maintained as monolayers.

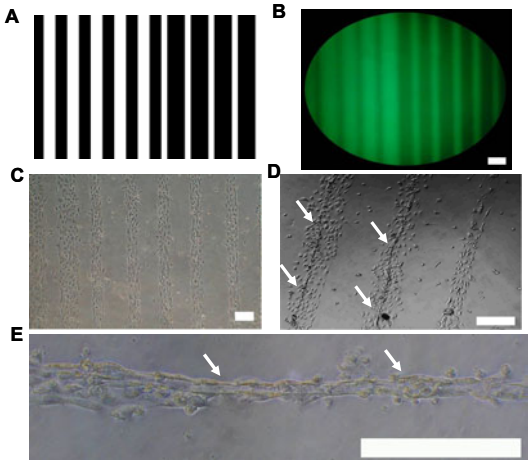

Fig 2. Patterning on PEG hydrogels. A) Blank stripes used as the pattern. B) Visualization of patterned stripes with FITC-conjugated PEG. HUVEC adhesion on RGDS patterns after C) 1 day and D) after 10 days in culture. E) HUVECs formed tube-like structures in the middle of the 70 μm wide lines. White arrows indicate tube-like structures. Scale bar = 200 μm

Confocal microscopy images indicate that HUVECs in the center of the stripes begin to protrude their nuclei and cell bodies vertically upward as soon as 2 days in culture (Fig 3B) and eventually coalesce together to form tubules with lumens. (Fig 3C) However, cells in the periphery of the patterned areas remain spread.

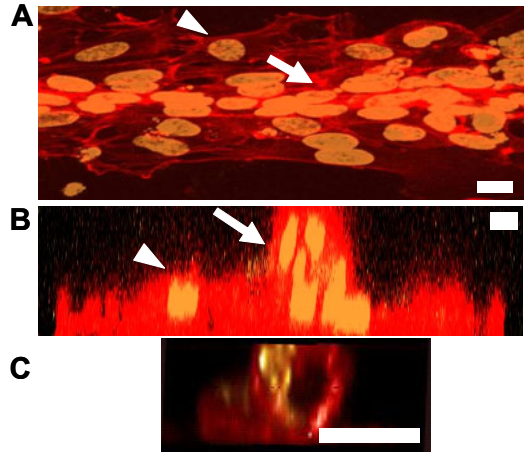

Fig 3. Confocal images of A) horizontal and B), C) vertical cross section of tube-like structures formed by HUVECs. Phalloidin-TRITC and DAPI are shown in red and orange, respectively. Arrows and arrow heads indicate HUVECs located in the periphery and center of the patterned stripes, respectively. Scale bars = 10 μm.

1. Dike, LE, et al. In Vitro Cell Dev Biol Anim. 35 (1999) 441-8.
2. Co, CC., et al. Am Chem Soc.127 (2005) 1598-9.
3. Hahn, MS., et al. Biomaterials. In Press. (2005).