Modifications of the PEGDA hydrogels to regulate endothelial angiogenesis and vasculogenesis

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Introduction

Angiogenesis and vasculogenesis are two modes of morphogenesis undertaken by endothelial cells during new blood vessel formation [1]. These categories of vessel formation have been traditionally studied on natural extracellular matrix proteins. In this work, we demonstrate that both categories of blood vessel formation can be recapitulated on synthetic biomaterials, namely bioactive poly(ethylene glycol)-diacrylate (PEGDA) hydrogels that provide more control over the presentation of biomolecules. The results presented here demonstrate the efficacy of peptide-modified PEGDA hydrogels as a scaffold material to promote two categories of vessel formation and suggest future applications of these materials to orchestrate and recapitulate tissue complexity and functions.

Materials and Methods

PEGDA (MW 6000) was synthesized by reacting PEG with acryloyl chloride in the presence of triethylamine. A proteolytically degradable PEG precursor was synthesized by conjugating acrylate-PEG moieties to the collagenase-sensitive peptide sequence, GGGLGPAGGK. A highly potent angiogenic factor, ephrin-A1, and a cell adhesion peptide RGDS were coupled to PEG monoacrylate by reaction with acryloyl-PEG-N-hydroxysuccinimide (MW 3400). PEG conjugation was confirmed by gel permeation chromatography and SDS-PAGE followed by Commassie staining. The acrylated peptides and proteins were immobilized on the surface of PEG hydrogels via two photopolymerization steps. First, the base polymer was photopolymerized with PEGDA (MW 6000). Then the prepolymer solution containing the acrylated peptides and proteins were applied and photopolymerized on the surface of the base polymer to create thin hydrogel films. To pattern RGDS on the hydrogels, a transparency mask with desired patterns was laid on top of the second precursor solution during photopolymerization. Human umbilical vein endothelial cells (HUVECs) were seeded on the hydrogels, and at various time points, the HUVECs were fixed, stained with phalloidin-TRITC and DAPI, and visualized with confocal microscopy. To investigate endothelial angiogenic response in 3D, HUVECs were encapsulated at a density of $1.5X10^6$ cells/ml in proteolytically degradable PEGDA hydrogels. After 2 days in culture, the cells were visualized by confocal microscopy after staining with phalloidin-TRITC and DAPI.

Results and discussion

HUVECs seeded on hydrogels with immobilized ephrin-A1 and RGDS (both 2.0 μg/cm^2) underwent tubulogenesis, a process intimately associated with angiogenesis and formed capillary-like structures with luminal diameters ranging from 5 – 30 μm after 9 days in culture (Fig 1). Ephrin-A1 enhanced cell adhesion, increased the total length of tubes formed, and acted independently from the RGDS concentration (2.0 and 20.0 μg/cm^2).

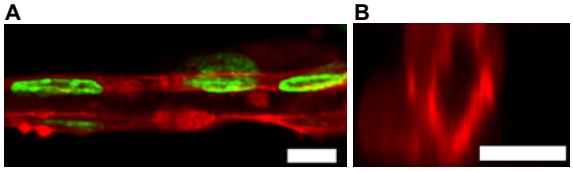

Fig 1. A) Horizontal and B) vertical images of capillary-like structures of HUVECs after 9 days on PEGDA hydrogels with RGDS and ephrin-A1 each at 2.0 μm/cm^2. Phalloidin-TRITC and DAPI are shown in red and green, respectively. Scale bars = 10 μm.

Furthermore, we aimed to guide endothelial tubule formation geometrically by micropatterning RGDS in desired shapes on 2D surface of hydrogels using a transparency-based photolithography. HUVECs reorganized their cell bodies into tubular structures reaching several mm in length on 50 μm but not on 200 μm wide stripes of RGDS, suggesting that confining distribution of the cell binding ligands stimulates angiogenesis. Confocal microscopy images indicate that HUVECs in the center of the 50 μm wide stripes begin to protrude their nuclei and cell bodies vertically upward as soon as 2 days in culture (Fig 2A) and eventually coalesce together to form tubules with lumens (Fig 2B). However, cells in the periphery of the patterned areas remain spread. Next, we aimed to promote another mode of vessel formation, i.e. vasculogenesis, by culturing HUVECs in 3D PEGDA hydrogels designed to be degraded by proteases. HUVECs encapsulated in collagenase-sensitive hydrogels initiated the first step of vasculogenesis and formed large internal vacuoles devoid of cytoskeletons or nuclei as visualized by confocal microscopy (Fig 2C).

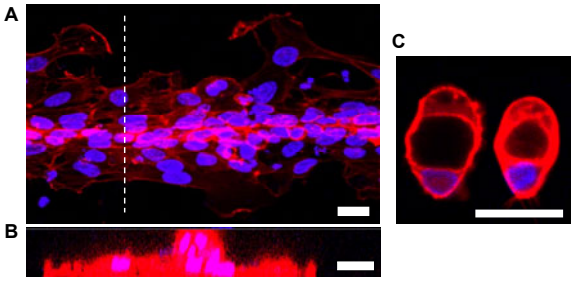

Fig 2. A) Confocal image demonstrating presence of densely coalesced, cord-like structure along the central axis of the stripe. B) Vertical cross-section along the dashed line in A) shows protrusion of some population of ECs to participate in tubulogenesis. C) A confocal optical section showing HUVECs undergoing vasculogenesis in the hydrogels. Cells stained with TRITC-phalloidin and DAPI are shown in red and blue, respectively. Scale bars = 20 μm

Acknowledgements

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1. Davis, G.E., et al. (2002) Anat Rec. 268, 252-75.