Modification of biomaterials with ephrins and Eph ligands for angiogenic applications

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Introduction

Recent developments in Eph/ephrin research suggest a large potential for therapeutic applications. Interplay between Eph-ephrin plays major roles in neuronal guidance, vasculature development, and angiogenesis. Specifically, we chose to work with ephrin-A1 and EphB4 to promote angiogenesis in the tissue engineered construct as they have been found to induce strong angiogenic responses in endothelial cells [1, 2]. In this report, we describe novel applications of ephrin-A1 and EphB4 to cardiovascular engineering using polyethylene glycol (PEG) hydrogels as a bioinert base material.

Materials and Methods

PEG-diacrylate (MW 6000) was synthesized by reacting PEG with acryloyl chloride in the presence of triethylamine. Ephrin-A1 and EphB4 (R & D systems) were coupled to PEG monoacrylate by reaction with acryloyl-PEG-N-hydroxysuccinimide (MW 3400). The cell adhesion peptide RGDS was conjugated to PEG monoacrylate in a similar manner. PEG conjugation was confirmed by gel permeation chromatography and SDS-PAGE followed by Commassie stain. The acrylated peptides and proteins were immobilized on the surface of PEG hydrogels via two photopolymerization steps. First, the base polymer was photopolymerized with PEG-diarylate (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. Human umbilical vein endothelial cell (HUVEC) adhesion and spreading were evaluated both on PEG hydrogels and polystyrene wells presented with various concentrations of ephrin-A1 or EphB4. In competitive inhibition studies, soluble inhibitors (RGDS, RGES, $\alpha_v\beta_3$, $\alpha_v\beta_5$ antibodies) were added in media during cell attachment. After 9 days of culture, HUVECs were fixed, stained with phalloidin-TRITC and DAPI, and visualized with confocal microscopy.

Results and Discussion

To present ephrinA1 and EphB4 in a highly controlled system, these proteins were immobilized on the surface of PEG-diacrylate hydrogels by photopolymerization along with RGDS (2.0 μg/cm^2). RGDS was required to induce EC adhesion as neither ephrinA1 nor EphB4 alone supported cell adhesion. EphrinA1 immobilized on hydrogels promoted a dose-dependent increase in EC adhesion and spreading while EphB4 stimulated their adhesion and spreading in a bi-phasic manner (Fig 1). Similar findings were observed with ECs seeded onto polystyrene wells adsorbed with ephrinA1 or EphB4, indicating maintenance of their bioactivities following PEG conjugation and incorporation on hydrogel surfaces.

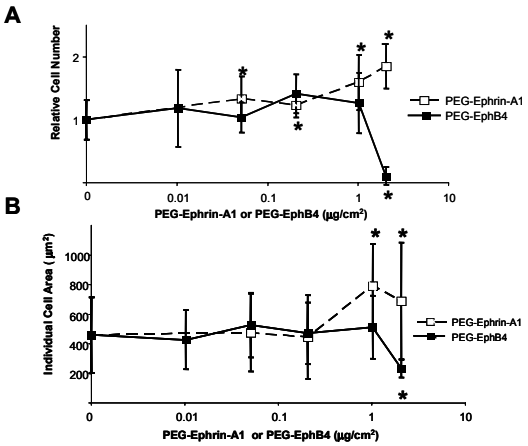

Figure 1. HUVEC A) adhesion and B) spreading on PEG hydrogels immobilized with ephrin-A1 or EphB4

HUVEC adhesion stimulated by ephrin-A1 and EphB4 was abolished by treatment with soluble RGDS and anti-$a_v\beta_3$ integrin but not anti-$a_v\beta_5$ integrin antibody. These data suggest that ephrin-A1 and EphB4 mediate cell adhesion through $a_v\beta_3$ integrins. After 9 days in culture, HUVECs formed capillary tube-like structures with luminal diameters ranging from 5 – 30 μm on hydrogels immobilized with ephrin-A1 and RGDS as visualized with confocal microscopy (Fig 2).

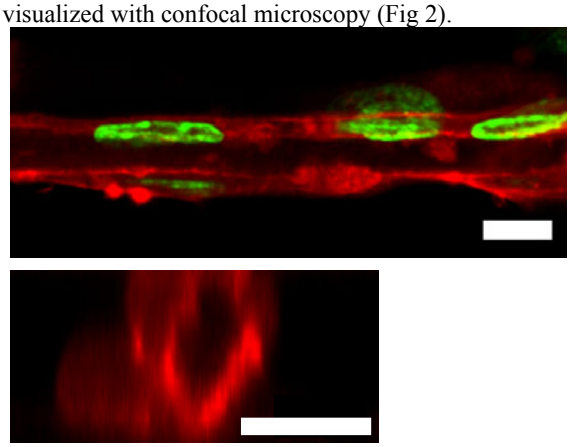

Figure 2. Capillary-like structures of HUVECs after 9 days on PEG hydrogels with RGDS and ephrin-A1 each at 2.0 μm/cm^2. Phalloidin-TRITC and DAPI are shown in red and green, respectively. Scale bars = 10 μm.

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). The current system is highly conducive to angiogenesis, and these findings suggest potential applications of ephrin-A1 and EphB4 in vascularization of tissue engineered constructs.

Acknowledgements

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1.Brantley-Sieders et al. J Cell Sci. 117 (2004) 2037-49.
2. Fuller et al. J Cell Sci. 116 (2003) 2461-70.