Biomaterial Topography Promotes Endothelial Cell Migration via Disrupted VE-Cadherin Signaling

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Statement of Purpose: Small-diameter prosthetic vascular grafts have unacceptably low patency rates. This is partly attributed to the inability of the neighboring, mature endothelial monolayer to migrate across the anastomosis and endothelialize the graft luminal surface.[1] Confluent, endothelial cells (EC) form strong cell-cell junctions that induce quiescence via contact inhibition; thus, we aimed to design a biomaterial capable of activating EC monolayers into a proliferative and migratory state. Previous reports suggest that biomaterial topography can alter aspects of EC morphology, including the actin cytoskeleton.[2] As the actin network influences cell-cell junction integrity, we hypothesized that altering fibrous topography of an elastin-like protein (ELP) fabric could also influence cell-cell junctions and hence activate EC monolayers to become migratory and proliferative.

Methods: The modular ELP design consists of 4 alternating repeats of a fibronectin-derived RGD sequence and a structural elastin-like domain.[3] Recombinant ELP was expressed in *Escherichia coli*, purified, electrospun, and crosslinked into stable fibrous matrices with diameters of 0.8, 1.2, or 2.0 μm (Fig. 1A). Matrices were characterized by confocal and scanning electron microscopy and tensile strain. The ELP sequence includes a fibronectin-derived RGDS cell-adhesive ligand (18 RGDS/1000 μm^2). Final mechanical properties were tuned via amine-targeted crosslinking chemistry (tensile moduli ~150 kPa). Human umbilical vein endothelial cell (HUVEC) monolayers cultured on the matrices were assessed by immunocytochemistry for key cellular structures (vinculin, actin cytoskeleton, VE-cadherin junctions) and proliferation markers (Ki67), by time-lapse microscopy for analysis of collective cell motility, and by Western blotting for FAK and ERK1/2 activation.

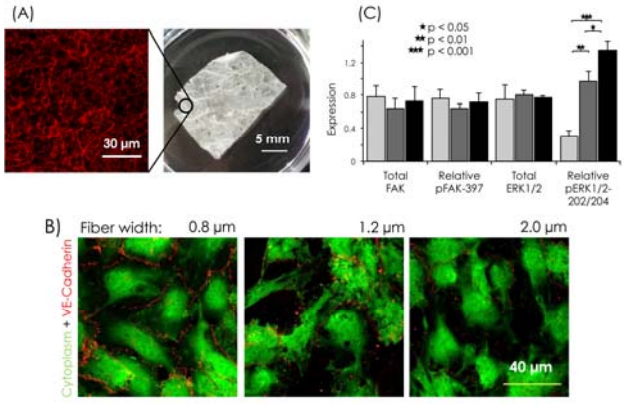

Figure 1. (A) Confocal microscopy and photograph of fibrous ELP matrix. (B) VE-cadherin immunostaining of HUVEC monolayers. (C) Western blot analysis of p/FAK and p/ERK1/2.

Results: VE-cadherin is a master regulatory protein for homotypic cell-cell junctions in ECs. On wider diameter fibers, localization of VE-cadherin at cell-cell junctions was significantly decreased (Fig. 1B), thus validating our hypothesis that ELP topography could influence cell junction morphology. Furthermore, the fraction of cells undergoing proliferation (i.e. Ki-67 positive) was maximal on intermediate sized fibers, consistent with the reported mitogenic effects of cadherin.[4] Phosphorylation of ERK1/2, which drives integration of mitogenic and migratory signals from the extracellular matrix, was higher for HUVEC seeded onto wider fibers (Fig. 1C). Collectively, these data suggest that a loss of cell-cell junction integrity on wider diameter matrices results in a more proliferative cell state. Time-lapse microscopy of endothelial monolayers indicated that HUVEC on wider diameter fibers migrated at higher speeds, but with less persistence (Fig. 2). Additionally, the migration paths of individual ECs were found to be less correlated with the migration paths of their neighbor cells on wider diameter fibers. These effects are consistent with a loss of cell-cell signaling via VE-cadherin. HUVEC monolayers treated with a VE-cadherin-blocking antibody were no longer able to respond to changes in biomaterial topography, confirming the role of cell-cell signaling in this system.

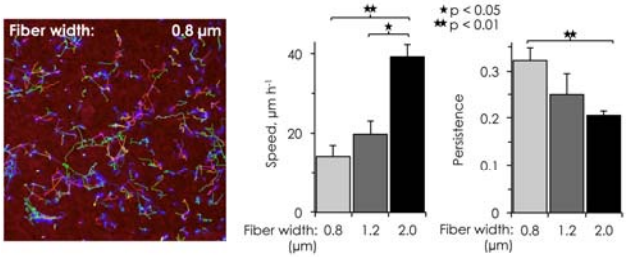

Figure 2. (A) Tracks of individual cells followed by time-lapse microscopy. (B) Quantification of cell speed and persistence.

Conclusion: The ability of the endothelium to cross into the anastomosis site is critical for long-term patency of short-diameter vascular grafts. A proliferative and migratory monolayer is needed for adequate endothelialization of the graft, but the native endothelium is limited by contact inhibition-induced quiescence. Here, we establish that cell-cell junction morphology can be controlled through tuning of electrospun ELP topography. Disruption of VE-cadherin junctions was directly implicated in increased proliferation and enhanced motility speed. Collectively, these data indicate that a monolayer-wide, phenotypic shift away from quiescence can be induced by simply altering biomaterial topography. These results reinforce the notion that cell-scale, fibrous topography is a critical design parameter for implantable biomaterials and may promote endothelial cell activation.

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