Micropatterns Enhance Endothelial Cell Migration Under Flow Conditions

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Statement of Purpose: Cardiovascular disease remains the leading cause of mortality among adults in the US. As a result, nearly 600,000 coronary and peripheral vascular bypass graft surgeries are performed in the US each year.[1] While autografts are the current gold standard for replacing small diameter blood vessels (< 6 mm), this treatment leads to significant drawbacks, including limited availability and donor site morbidity. Synthetic grafts have also been unable to address this problem, as shown through their poor patency rates and lack of vascular regeneration in small-diameter vessels.[2] Synthetic grafts commonly fail due to thrombosis or intimal hyperplasia, which occur as a result of the absence of endothelial cells lining the lumen of the graft.[3] To overcome these limitations, Sharklet Technologies, Inc. (STI) proposes a tissue engineered vascular graft comprised of a Sharklet micropatterned acellular extracellular matrix to enhance graft incorporation via guided endothelial cell migration onto the graft lumen.

Methods: The first phase of STI's graft enhancement was to evaluate endothelial cell migration across the Sharklet micropattern under both static and flow conditions. Three Sharklet patterns of varying size (+1SK2x2, +1SK10x5, +10SK50x50) were replicated in poly(dimethylsiloxane) elastomer (PDMSe). Squares (1 cm x 1 cm) of each pattern and smooth were attached to a silanized glass slide in randomized order with features oriented parallel to the long axis. Once sterilized, slides were coated with 43µg/cm^2 collagen and incubated for 1 h (37° C, 5% CO_2). Samples were rinsed with phosphate buffered saline, then coated with 3.2 µg/cm^2 fibronectin and incubated overnight (37° C, 5% CO_2). After aspirating the fibronectin, a 1 cm x 3 mm rectangle of smooth PDMSe was placed across each patterned square to simulate a cell-absent region on a graft. Once dry, slides were seeded with human coronary artery endothelial cells (HACECs, ATCC PCS-100-020) and maintained in growth media and incubated until reaching 80-90% confluency.

To evaluate migration under static conditions, the smooth PDMSe rectangles were removed to allow cells to migrate over the course of 3 days. For laminar flow conditions, rectangles were removed and slides were placed in a parallel plate flow chamber. The test ran for 24 h, with a flow rate of 145 ml/min (37° C, 5% CO_2) that applied 5 dyn/cm^2 of pulsatile shear across three slides. At the conclusion of each test, cells were stained with Cell Tracker Orange CMTMR Dye (ThermoFisher) and fixed in 4% paraformaldehyde. Slides were imaged under a fluorescent microscope and analyzed for percent coverage across the migration region.

Results: Results from static migration showed all three patterns significantly improved cell migration compared to smooth (Dunnett's Test, Fig 1). The +10SK50x50 micropattern resulted in the highest level of increased migration (40%, p=0.01). Assays conducted under laminar flow revealed differences in percent area coverage for the various Sharklet micropatterns (Fig 1.) Both +1.5SK10x5 and +10SK50x50 increased cell migration significantly compared to smooth, 139%, p=0.05 and 181%, p=0.01, respectively.

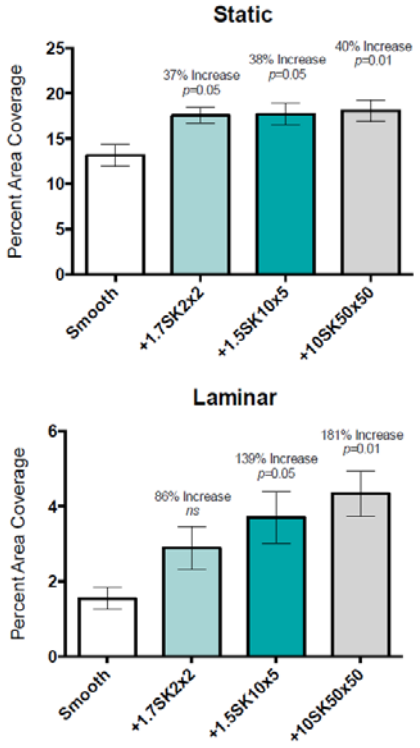

Figure 1. Cell Coverage after static (top) and Laminar slow (bottom) migration assays. Results show that Sharklet micropatterns can significantly increase HCAEC migration under both static and laminar flow conditions.

Conclusions: Sharklet micropatterns significantly increases cell migration under both static and laminar flow conditions. Collectively, these results provide motivation for continued research by demonstrating Sharklet micropatterns may improve graft incorporation via guided endothelial cell migration under physiologically relevant conditions.

References:

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