Engineered Microtopographies Direct Human Coronary Artery Cell Elongation and Orientation

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Statement of Purpose: The clinical application of synthetic small-diameter vascular grafts has been limited due to high rates of occlusion from thrombosis and intimal hyperplasia. Smooth muscle cells (SMCs) can shift reversibly from a quiescent, contractile phenotype to a synthetic phenotype. The synthetic phenotype is characterized by proliferation and extracellular matrix synthesis. During intimal hyperplasia a shift of SMCs to the synthetic phenotype results in proliferation of SMCs and narrowing of the blood vessel. Strategies such as topographically modifying cell culture substrates have been investigated for controlling SMC phenotype. Microchannel scaffolds with discontinuous walls initially supported primary vascular smooth muscle cell proliferation in the synthetic phenotype. Upon reaching confluence, the cells were aligned and transformed towards the contractile phenotype[1]. This evidence supports the hypothesis that mechanical forces such as those experienced by cells grown on topographies can regulate gene expression and may act to prevent dedifferentiation of SMCs into the phenotype that causes intimal hyperplasia. Topographies ranging from the nanoscale to large microtopographies, like the microchannel scaffolds, that confine cells to a particular shape have been investigated for phenotype control. The approach in this work is to create engineered microtopographies that allow cells to spread across but are not physically constrained by the surface to influence cell elongation, orientation and differentiation.

Methods: Free-standing films of smooth and topographically modified Silastic® T2 polydimethyl siloxane elastomer (PDMSe) were produced. The elastomer was prepared by mixing 10 parts by weight of resin and 1 part by weight curing agent. The mixture was stirred by hand for 5 min and degassed under vacuum (28-30 in Hg) for 30 min to remove bubbles. The elastomer was polymerized in a silicon wafer mold at ambient for 24 h. The topographies tested included the n-series of Sharklet AF™ topographies and channels with a height of 1 µm and width and spacing of 2 µm. The n-series is a group of topographies designed to have an increasing number of unique features (n) arranged in the Sharklet AF™ pattern (Figure 1). The n-series surfaces were referred to as +1SK2x2_n4 where n is the number of unique features and ranges from one to five. The channels topography was represented as +1CH2x2. Three 14 mm discs of each film were punched out and attached to wells of a 24-well plate with uncured polymer. The samples were sterilized by immersion in 70% v/v ethanol in water for 1 h. Samples were then rinsed with PBS three times and immersed in 50µg/mL fibronectin in PBS overnight. Human coronary artery endothelial cells (ECs) or SMCs were seeded onto samples at $2.5x10^4$ cells/well and placed into an incubator at 37°C with 5% CO_2. Samples were imaged after 24 h and 7 d using an inverted phase contrast microscope. Three images were taken at 10x magnification for each topography. Cell elongation and orientation were quantified using ImageJ software. Elongation was quantified by calculating the cell shape index (CSI) for each cell. The CSI ranges from 0 (elongated, linear cells) to 1 (circular shaped cells).

Results: The Sharklet AF™ topographies with the longest feature lengths (n=4,5) in the n-series and continuous channels resulted in the largest elongations and orientations of ECs and SMCs after 24 h and 7 d. Cells cultured on the smooth PDMSe standard and TCPS control did not show preferential orientation, i.e., the mean angles on these topographies were approximately 45°. After 7 d the highest degree of orientation was observed on +1SK2x2_n5 for ECs. More ECs were orientated along the direction of the channels on each topography after 7 d than after 24 h. The highest degree of orientation was observed on +1SK2x2_n4 for SMCs after 24 h and 7 d.

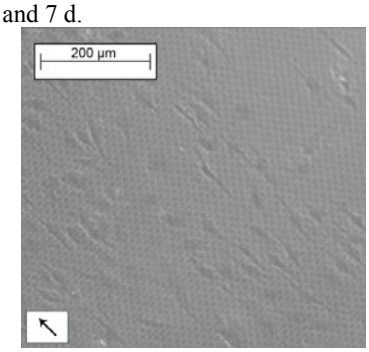

Figure 1. Phase contrast image of SMCs on +1SK2x2_n3 after 7 d. Arrow indicates direction of channels in the topography.

Conclusions: Microtopographies directed alignment and elongation of both human coronary artery cell types without physically constraining the cells. Topography can also trigger the expression of the contractile phenotype in SMCs[1-3]. Using topography to direct SMCs to express the contractile phenotype could be a strategy to reduce intimal hyperplasia in small diameter vascular graft applications. Protein expression of SMCs will be quantified in cells cultured on both topographically modified and smooth PDMSe to determine if any of the n-series topographies could be used to regulate not only orientation, but also phenotype of these cells.

References:

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