Smooth Muscle Interaction with Collagen Immobilized Nanowire Surfaces

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Statement of Purpose: Smooth muscle cells (SMCs) are key players in the development of vascular disease, a major concern worldwide. The standard treatment for vascular disease is coronary angioplasty, which leads to disorder of the endothelial layer. This leaves a highly prothrombotic surface exposed to the blood stream and promotes SMC proliferation. Restoration of an intact endothelium, therefore, represents a crucial process in re-establishing an intact vessel surface, but in order to do this SMC proliferation must be controlled. Enhanced SMC proliferation often leads to restenosis, the reocclusion of the blood vessel. SMCs are remarkably plastic and can easily dedifferentiate into the synthetic phenotype, allowing them to proliferate. Therefore, it is important that SMCs retain their differentiated (quiescent) phenotype on implant surfaces to reduce proliferation.

A popular approach for controlling cell adhesion and differentiation to implant surfaces is the introduction of surface topographies in the micrometer or in the nanometer range. Further, cell-substratum interactions are central to many biological phenomena and knowledge of these interactions is central to the design of blood contacting surfaces.

Methods: Flat polycaprolactone surfaces (PCL) were fabricated by sintering polycaprolactone pellets. Nanowires (NW) were fabricated using the established solvent free nano-templating technique. Collagen was immobilized to the PCL and NW surfaces utilizing aminolysis (notation: cPCL, cNW). Surfaces were characterized via contact angle measurements and scanning electron microscopy. HASMC functionality in terms of adhesion, proliferation, elongation and viability was investigated for up to 7 days in culture on collagen immobilized surfaces as well as non-immobilized surfaces using fluorescence microscope imaging, a cell viability assay and scanning electron microscopy. Differentiation was investigated at day 14 with immunofluorescence, staining for HASMC specific differentiation markers: heavy chain myosin (MYH) and calponin (CAL).

Results: Surface characterization results revealed surface characteristics were unaltered after collagen immobilization (Figure 1). Contact angle measurements decreased with the immobilization of collagen, indicating an increase in surface energy. Fluorescent microscopy results reveal an increase in HASMC adhesion on cPCL and cNW surfaces in comparison to PCL and NW surfaces after 1 and 7 days in culture. Results also reveal a significant decrease in cell elongation on PCL/cPCL versus NW/cNW surfaces. HASMC viability was measured using a commercially available MTT assay. Results show a significant decrease in cell metabolism on nanostructured surfaces (Figure 2). The viability of HASMCs is greatest on PCL and cPCL surfaces after 1, 4 and 7 days of culture. SEM results indicate more cell coverage on cPCL and cNW surfaces after 1 and 7 days of culture. Further, HASMC extracellular matrix deposition and cell extensions are present on NW, cPCL and cNW surfaces. HASMC differentiation studies after 14 days of culture reveal an increase in both MYH (Figure 3) and CAL expression on NW and cNW surfaces in comparison to PCL and cPCL surfaces.

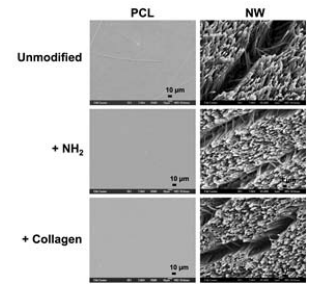

Figure 1. Surfaces before and after modification

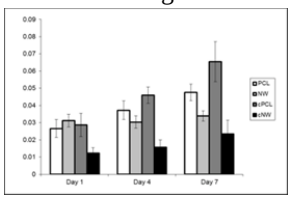

Figure 2. MTT results for HASMC viability

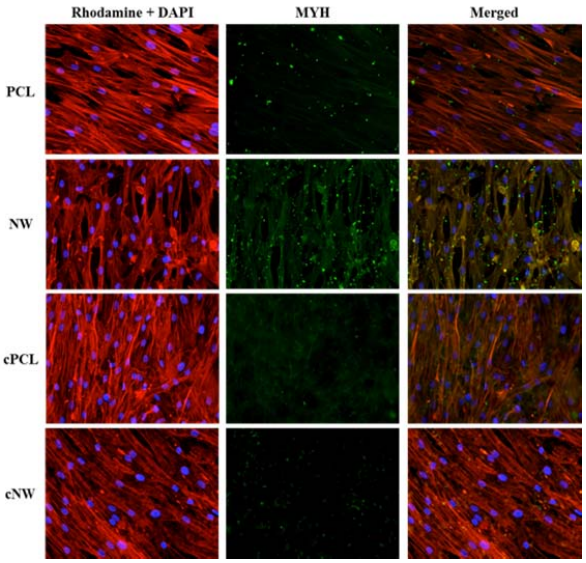

Figure 3. Representative immunofluorescent images of HASMCs after 14 days in culture on different surfaces, stained for MYH.

Conclusions: Maintaining a quiescent SMC state is essential for the health of human vasculature. Cell elongation is an important marker of a SMC differentiated cell state. HASMC elongation is greatest on NW and cNW surfaces. Cell viability is lowest on these surfaces. This may be due to the fact that the cells are no longer dividing and are in a quiescent state. Immunofluorescent results confirm this by showing an increased amount of both CAL and MYH expression on NW and cNW surfaces. Therefore both NW and cNW surfaces may be important surfaces to investigate for cardiovascular applications.

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