A Novel Endothelial Cell Scaffold for Small-Diameter Vascular Engineering

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Statement of Purpose: Cardiovascular disease (CD) accounts for approximately 35% of deaths in the United States and is the leading cause of both morbidity and death worldwide.[1] The current standard of care for critical CD is a bypass that uses relocated autologous vessels to provide an alternative path for blood in the location of a failing vessel. Though this strategy can be quite effective, many patients do not have vessels suitable for this procedure due to previous harvesting or systemic vascular pathology. For these patients, artificial grafts must be used. While poly(ethylene terephthalate) and poly(tetrafluorethylene)-based grafts have experienced great success as large vessel substitutes, there are currently no artificial replacement strategies successful for small-diameter (<6mm) vessels because of the additional challenges presented by a low-flow environment. Artificial small-diameter vascular grafts used in the past have failed largely due to stenosis or thrombosis that causes loss of patency. However, cell-based approaches may be able to prevent these issues by covering the graft surface to prevent direct biomaterial-blood contact and present a more physiologically-relevant intima. This study investigates the use of a porous thin film scaffold with minimal topography to support endothelialization for small-diameter vessel tissue engineering.[2]

Methods: Pore casting is a novel, robust scaffold fabrication technique that induces pores into thin films. Briefly, photolithography and reactive ion etching were used to create a silicon master mold with sub-micron features. Spin-assisted polymer templating was then used to deposit a thin layer of poly(caprolactone) (PCL) onto the mold. The PCL film was then peeled off to reveal a scaffold with full-thickness pores. Human umbilical vein endothelial cells (HUVECs) were cultured on the scaffold for 2 weeks and compared to cells on electrospun meshes.

Results: HUVECs displayed similar levels of adhesion on electrospun and pore-cast scaffolds with high plating efficiencies of 97.1 ± 0.3% and 97.2 ± 0.6% respectively ($p=0.85$). However, all other measures of cell behavior indicated enhanced endothelialization on pore-cast scaffolds compared the electrospun controls. HUVECs on the electrospun PCL mesh appeared stretched between adjacent fibers and failed to cover the surface of the scaffold when viewed using scanning electron microscopy. These cells also displayed limited tight junction assembly, intense staining for longitudinal f-actin, and cell nuclei in multiple focal planes suggesting a layered structure uncharacteristic of normal healthy endothelium. Alternately, cells on pore-cast PCL were flat and fully covered the scaffold surface. In addition, immunohisochemical staining of HUVECs on pore-cast PCL revealed cortical f-actin and superior tight junction formation, two markers of a mature endothelium. This drastic difference was further supported by transepithelial resistance which measured 13±2 Ω/cm^2 on pore-cast PCL compared to 1±1 Ω/cm^2 ($p<0.001$).

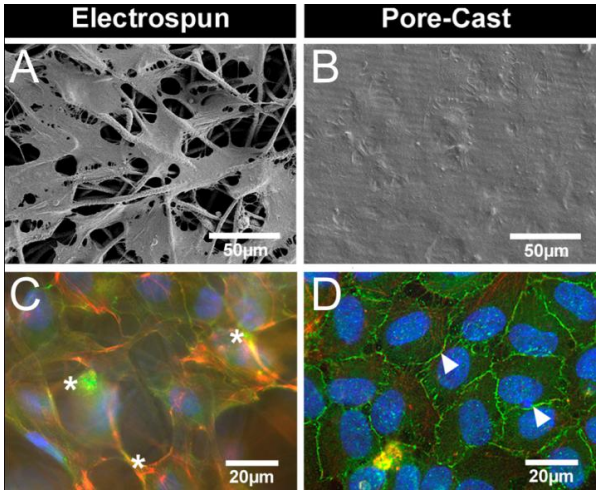

Figure 1. HUVECs cultured on electrospun and pore-cast PCL scaffolds. Scanning electron microscopy images of cells on (A) electrospun and (B) pore-cast PCL. Immunohistochemical staining of cells with ZO-1 (green), f-actin (red), and DAPI (blue) on (C) electrospun and (D) pore-cast PCL. Asterisks and arrowheads indicate intense f-actin and ZO-1 localization respectively.

Conclusions: Endothelial cells on pore-cast scaffolds exhibited superior morphology, protein localization, and functional barrier formation compared to cells on electrospun controls. Further, these data indicate that pore-cast scaffolds are able to promote the formation of a high-functioning endothelium that is likely critical for maintaining patency in small-diameter engineered vessel. Future experiments will determine the ability of the engineered endothelium to prevent undesirable platelet aggregation and withstand physiological flow. The vision for this research is ultimately to pair endothelial cells on pore-cast films with smooth muscle cells in an electrospun mesh to form a bi-layered scaffold that could be rolled into a cylinder and implanted as an artificial small-diameter vascular graft.

References: [1]Lloyd-Jones, et al., Circulation 2009;119: e21-181. [2]Isenberg, et al., Circ Res 2006;98:25-35.

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