Interaction of Endothelial Cells and Smooth Muscle Cells with ePTFE and Electrospun PTFE

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Statement of Purpose: Each year, more than 1.4 million people undergo arterial bypass surgery in the United States. Out of this, one-third of the patients require synthetic vascular grafts [1]. Expanded PTFE (ePTFE) is one of the currently used biomaterials for vascular grafts [1, 2]. ePTFE has shown successful outcomes when it is used for making large-diameter vascular grafts (> 6 mm). However, the use of these materials for making small-diameter vascular grafts (< 6 mm) results in thrombosis and neointimal hyperplasia (due to smooth muscle cell growth) [1] The main reason for the occurrence of thrombosis in small-diameter vascular grafts is the lack of endothelialization [3]. Electrospun PTFE may assist the growth of endothelial cells because of its nanofibrous structure. In this study, the interaction of endothelial cells (ECs) and smooth muscle cells (SMCs) with ePTFE and electrospun PTFE was studied to determine which type of PTFE might be suitable for small-diameter vascular grafts.

Methods: The ePTFE and electrospun PTFE films (1 cm × 1 cm) (Zeus, USA) were chemically cleaned by immersing in ethanol for 2 min followed by 24 h drying in air. The specimens were characterized using contact angle, Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) to determine the surface wettability, chemical composition, and morphology, respectively. Human aortic endothelial cells (HAECs) and human aortic smooth muscle cells (HASMCs) were individually seeded on ePTFE and electrospun PTFE specimens at a cell density of 15×10^3. The viability and proliferation of cells were quantitatively measured at 1, 3, and 5 days using a resazurin fluorometric assay. Fluorescence microscopy (FM) was used to image the cell morphology after staining the cells with fluorescein diacetate. A one-way analysis of variance (ANOVA) was performed to determine the statistical significance for difference at $p < 0.05$.

Results: The contact angles of 140 ± 1.1˚ and 142.3 ± 1.8˚ were obtained for ePTFE and electrospun PTFE, respectively. FTIR showed no differences in surface chemical composition between ePTFE and electrospun PTFE. SEM images showed that ePTFE has a structure with extensive nodes and fibrils whereas electrospun PTFE has randomly oriented fibers with fiber diameters ranging from 0.5 to 3 micrometers (Fig 1). More number of ECs adhered on ePTFE films than that of electrospun PTFE films (Fig 2A). However, the cells continued to proliferate on both the surfaces from day-1 to 5, with higher rate of proliferation observed for electrospun PTFE (Fig 2A,B). The FM images showed that ECs were well grown on both the surfaces with a typical polygonal shape and spreading morphology (Fig 2C-E). The adhesion of SMCs were significantly greater on electrospun PTFE than that of ePTFE (Fig 3A, Day-1). The SMCs continued to proliferate well on electrospun PTFE from day-1 to 5, while the cells were not proliferated at all on ePTFE (Fig 3A,B). The FM images also showed the extensive proliferation of SMCs on electrospun PTFE with its typical spindle shaped morphology. On day-5, the SMCs were fully confluent on electrospun PTFE (Fig 3E), whereas only very few cells with an uncharacteristic discoid shape were observed on ePTFE (Fig 3D). This clearly indicates that ePTFE does not support the growth of SMCs.

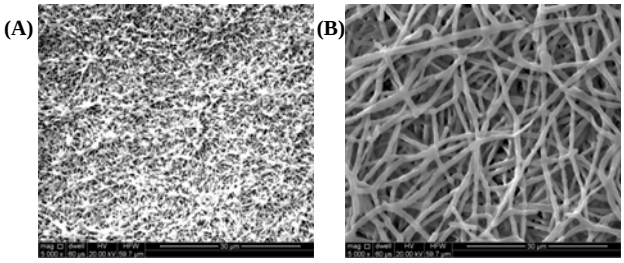

Fig 1. SEM images of ePTFE (A), and electrospun PTFE (B).

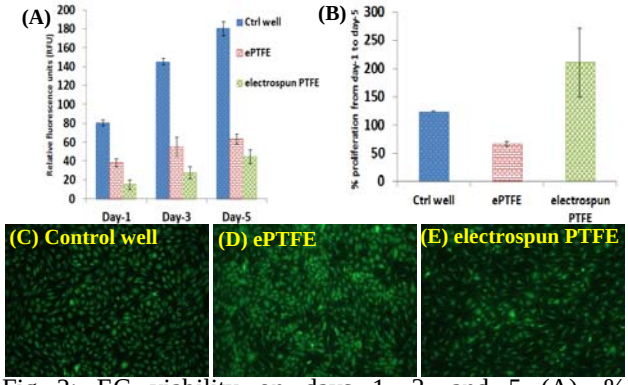

Fig 2: EC viability on days 1, 3, and 5 (A), % proliferation of ECs from day-1 to 5 (B), and morphology of ECs on day-5 (C-E).

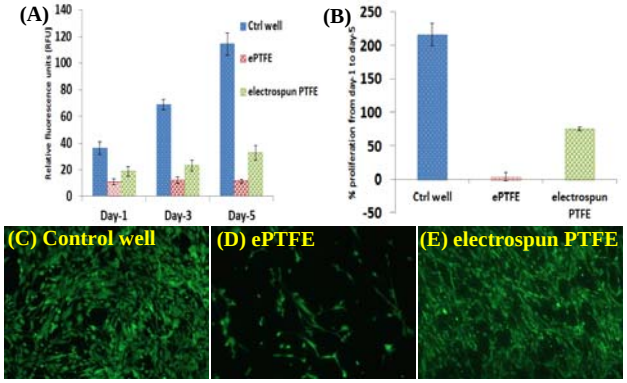

Fig 3: SMCs viability on days 1, 3, and 5 (A), % proliferation of SMCs from day-1 to 5 (B), and morphology of SMCs on day-5 (C-E).

Conclusions: Both ePTFE and electrospun PTFE encouraged the growth of ECs. However, only ePTFE inhibited the growth of SMCs.

References:

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