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Micropatterning of three-dimensional electrospun polyurethane vascular grafts

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ABSTRACT

The uniform alignment of endothelial cells inside small-diameter synthetic grafts can be directed by surface topographies such as microgrooves and microfibers to recapitulate the flow-induced elongation and alignment of natural endothelium. These surface micropatterns may also promote directional migration and potentially improve anastomotic ingrowth of endothelial cells inside the synthetic grafts. In this paper, we developed electrospinning and spin casting techniques to pattern the luminal surface of small-diameter polyurethane (PU) grafts with microfibers and microgrooves, respectively, and evaluated endothelial cell orientation on these surface micropatterns. Tracks of circumferentially oriented microfibers were generated by electrospinning PU onto a mandrel rotated at high velocity, whereas longitudinal tracks of microgrooves were generated by spin casting PU over a rotating poly(dimethylsiloxane) mold. We found that both PU grafts possessed longitudinal Young's moduli in the range of 0.43 ± 0.04 to 2.00 ± 0.40 MPa, comparable with values obtained from native artery. Endothelial cells seeded onto the grafts formed confluent monolayers with individual cells exhibiting elongated morphology parallel to the micropatterns. The cells were phenotypically similar to natural endothelium as assessed by the expression of the endothelial cell-specific marker, vascular endothelial cell cadherin. In addition, the cells were also responsive to stimulation with the pro-inflammatory cytokine tumor necrosis factor-α as assessed by the inducible expression of intercellular adhesion molecule-1. These results demonstrate that our micropatterned PU grafts possessed longitudinal Young's moduli in the same range as native vascular tissue and were capable of promoting the formation of aligned and cytokine-responsive endothelial monolayers.

1. Introduction

Cardiovascular disease is a major cause of death in the Western society [1,2]. More than one million vascular procedures, including bypass surgery, are performed each year in the USA alone [1]. The procedure typically requires the use of autologous veins (primarily saphenous veins) as bypass conduits to treat occlusion in coronary and peripheral arteries. This bypass surgery often results in secondary morbidity and is not possible in many patients who lack a suitable vein for bypass grafting [2–4]. Due to these limitations in autologous vessels, the use of synthetic vascular grafts has become an attractive "off-the-shelf" alternative as such grafts can be prefabricated to specific dimensions.

Despite clinical success for synthetic grafts with inner diameters larger than 6 mm, small-diameter synthetic grafts (<6 mm) often fail within a short time after bypass surgery due to thrombosis and intimal hyperplasia [2,4–7]. These problems are often caused by the lack of an endothelial coverage on synthetic grafts [8] and the mismatch between the mechanical properties [5,9,10] of synthetic materials and native vascular tissue. In the former case, the absence of endothelial cell ingrowth into the midgraft area can persist even after 10 years post-surgery [8]. Therefore, the development of compliant small-diameter synthetic grafts that promote endothelial cell adhesion is the central focus in most recent studies [4,5,7,11].

Electrospinning is a versatile method to construct three-dimensional (3D) synthetic polymeric grafts [2,4,5,7,12] with mechanical properties comparable to native vascular tissue [5]. In the electrospinning process, fibers with nanometer- to micrometer-scale diameters are generated when a jet of polymeric solution is drawn across an electric field and in the process solidifies on a rotating tubular collector [4,5,11,13]. Depending on the rotational speed of the collector, the electrospun fibers can be organized into a nonwoven mesh [5,7,14] or into oriented patterns [7,11,15]. In addition to the use of elastomeric polymers to match the compliance

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of native vascular tissue, the physical entanglement of polymeric fibers into a 3D matrix has been shown to further increase the grafts' compliance as well as contribute to the healing process [5].

The natural nonthrombogenic property of healthy blood vessels is conferred by endothelial monolayers that line the vessel lumens. Cell-surface interactions play a key role in the formation of endothelial monolayers on synthetic polymer surfaces. Endothelial cells have been shown to recognize and respond to the presence of micropatterns on their underlying substrata. Previous studies on synthetic surfaces have shown that surface topographies such as pores [1,16–18] and meshes [7,14] effectively stimulate spreading, adhesion and proliferation of endothelial cells, whereas oriented fibers [7] and grooves [6,19] induced a uniform cell alignment on the surface. In our previous work [20] we found that the grooved patterns induced the uniform alignment of bovine aortic endothelial cells as well as directional cell migration even in the presence of physiological shear stress. Thus, well-defined tracks such as microgrooves on the synthetic grafts' lumen can potentially improve the anastomotic ingrowth of endothelial cells and subsequent formation of an endothelial monolayer.

In this paper, we used electrospinning and spin casting to pattern the luminal lining of small-diameter polyurethane (PU) grafts with microfibers and microgrooves, respectively, and compared their ability to promote endothelial cell alignment as well as their contribution to the graft's compliance. The alignment of individual cells parallel to tracks of microfibers and microgrooves persisted at confluence on both micropatterned surfaces. In addition, PU grafts fabricated by either technique exhibited Young's moduli in the longitudinal direction similar to the values obtained from native aorta and could withstand a uniaxial strain of up to 300%. Lastly, the cells maintained their response to stimulation by tumor necrosis factor-α (TNF-α) as shown in the increase of intercellular adhesion molecule-1 (ICAM-1), a key indicator of inherent endothelial physiology. Collectively, the micropatterns investigated in our study can be applied to the fabrication of novel small-diameter synthetic grafts that are capable of guiding cellular alignment while maintaining quasi-endogenous responsiveness of the endothelial monolayer.

2. Materials and methods

2.1. Electrospinning of polyurethane

Tecothane, an aromatic polyether polyurethane, was obtained from Thermedics Inc. (Woburn, MA), purified and characterized as previously reported [21]. Solutions of 5% (w/v) PU were made for electrospinning by dissolving lyophilized PU in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma), with stirring for 2 h at 40 °C and then overnight at room temperature. This PU concentration was established in preliminary studies in our laboratory to generate continuous bead-free microfibers.

A horizontal electrospinning apparatus was setup inside a chemical hood as shown in Fig. 1. The power supply (ES30-0.1P, Gamma High Voltage Research) was set to 12 kV and the distance between the reservoir of the PU solution and the target that collected the electrospun fibers was kept constant at 12 cm. The PU solution inside a syringe was fed at 0.8 ml h^{-1} by an infusion pump (KD Scientific Infusion Pump, Fisher) through a blunt 18 gauge stainless steel needle towards the target. To investigate the optimal PU concentration that yielded continuous microfibers, glass coverslips were placed on the flat copper target to collect electrospun fibers.

To fabricate PU grafts with circumferentially aligned microfibers by electrospinning (electrospun grafts), the copper plate was replaced with a high-speed rotating drill (Dremel). An aluminum (Al) mandrel was machined to 4 mm in diameter and 5 cm in length by the Drexel University Instrumentation Shop. During electrospinning, the Al mandrel was set to rotate at 35,000 rpm while collecting electrospun fibers. About 2 ml of 5% PU solution was used to fabricate one graft. After drying inside a hood overnight, PU grafts were released from the mandrel. The fiber dimension as well as graft thickness were examined and measured by scanning electron microscopy (SEM).

For the fabrication of PU grafts with luminal lining of microgrooves, a mold consisting of microgrooves (3.6 μm channel × 3.3 μm ridge × 1 μm depth) was first fabricated on a silicon wafer and then transferred to poly(dimethylsiloxane) (PDMS; Robert McKeown, Inc., Branchburg, NJ), as previously described [6]. This micropatterned PDMS sheet (2.5 × 2.5 cm^2 and 200 μm thick) was wrapped around a mandrel with the microgrooves aligned parallel to the mandrel length and secured with nail polish to create a 3D mold with a grooved surface exposed to air. Approximately 1 ml of 3% PU solution was spun cast on the slowly rotating mandrel (50 rpm) under a high-intensity halogen lamp (300 W) for 10 min to transfer the microgroove patterns from PDMS to PU. Immediately following the spin casting process, the 1 ml of 5% PU solution was electrospun onto the mandrel while rotating at 50 rpm. The resulting grafts were referred to as hybrid grafts. Both luminal and outer surfaces were then examined by SEM.

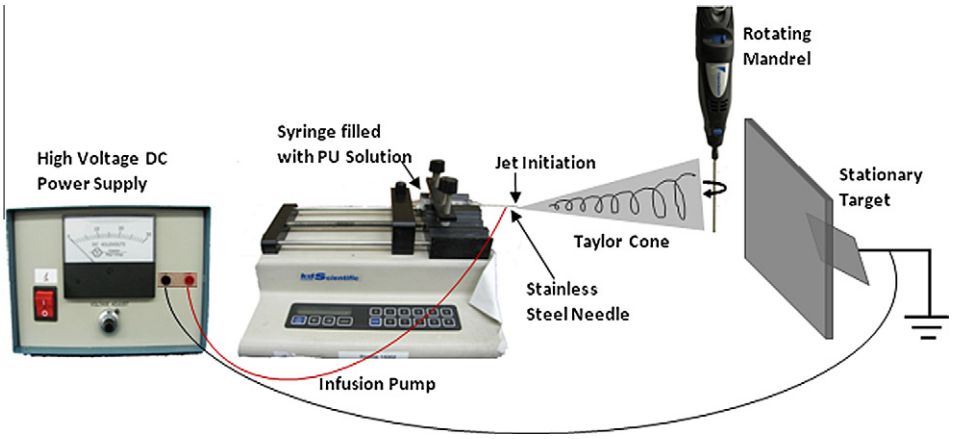

Fig. 1. Schematic of electrospinning setup. The electrospinning apparatus consists of (1) a high voltage DC power supply, (2) an infusion pump, (3) a syringe filled with PU solution that is attached to (4) a stainless steel needle, (5) a rotating mandrel and (6) a stationary target connected to the ground. The power supply generates an electric field between the needle and mandrel. The infusion pump drives a constant flow of PU solution, which forms a Taylor cone at the needle tip under the electric field, to solidify on the mandrel. For electrospun grafts, the mandrel is rotated at 35,000 rpm to generate circumferentially oriented microfibers along the mandrel. For hybrid grafts, a PDMS mold embedded with microgrooves is wrapped around the mandrel to replicate a microgroove pattern parallel to the mandrel's long axis onto the graft lumen.

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