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Vascular tissue engineering: microtextured scaffold templates to control organization of vascular smooth muscle cells and extracellular matrix

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Abstract

The *in vitro* construction of tissue-engineered small diameter (<6 mm) blood vessels with sufficient strength and mechanical compliance has evaded researchers. We hypothesize that the high spatial organization of the medial layer of vascular smooth muscle cells (VSMCs) and their surrounding matrix provides high burst strength, compliance, and stability. We investigated the effect of microfabricated polydimethylsiloxane (PDMS) scaffolds with various groove widths on VSMC organization. We found that the presence of these grooved topographical cues significantly enhanced VSMC aspect ratio, alignment, and oriented remodeling of the underlying extracellular matrix. This study suggests that topographical patterning of tissue scaffolds can influence cellular and matrix spatial organization and could provide a framework for achieving the required organization and physical properties for blood vessels.

Keywords: Microtopographical cues; Vascular smooth muscle cell alignment; Vascular tissue engineering; Fibronectin

1. Introduction

The development of tissue-engineered vascular grafts is critical not only for vessel replacement therapies but could also serve as model *in vitro* platforms for drug screening. A major goal in vascular tissue engineering, in particular small diameter (<6 mm) vessels, is to recapitulate the *in vivo* function of native blood vessels. While there has been much progress towards creating functional small diameter blood vessels *in vitro*, the structural integrity and vasoactivity of current engineered vessels are not yet comparable to that of native vessels [1–4]. It is also important to note that tissue-engineered vessels often lack the unique structural and hierarchical organization of cells and extracellular matrix (ECM) found in native blood vessels [1].

The medial layer provides the main structural support of the vessel (i.e. strength, elasticity, and contractility) and consists of multiple layers of vascular smooth muscle cells (VSMCs) and ECM that are arranged in distinct spiral configurations [5] (Fig. 1A). While the significance of this spiral organization is unclear, it is interesting to note that the helical pitch varies between species as well as in vessels with different functions [5]. In addition, the organization and structure of the ECM proteins such as fibronectin (FN), elastin and collagen play an important role in controlling the structural integrity of tissue engineered blood vessels [3]. We therefore hypothesize that the spatial arrangement of VSMCs and the ECM plays a critical role in the function of the medial layer.

Several attempts to tissue-engineer vascular constructs that mimic various aspects of the *in vivo* cellular

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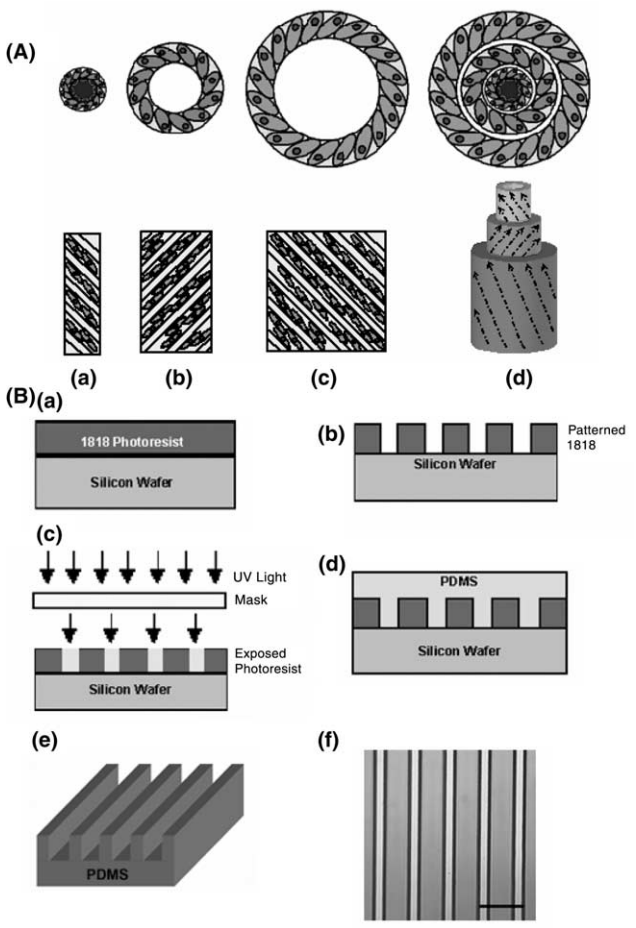

Fig. 1. (A) A schematic drawing of smooth muscle cell organization in the medial layer of elastic arteries based on structure proposed by Rhodin et al. [5]. VSMCs are arranged in spirals, which have a different pitch in different cylinders of the vessel. As viewed from the end (top panels), VSMCs are arranged at angles between two elastic laminae. If the cylinders are unrolled and observed in 2-D as a sheet of cells (bottom panels: (a)–(c)), it is seen that the cells are arranged in parallel strips across the sheets. Cells in (a) and (c) appear to have a clockwise arrangement when looking on end while a counterclockwise arrangement is seen in (b). Putting the three cylinders together as (a)–(c) as seen in (d) demonstrates how the herringbone pattern of VSMCs is formed. (B) Microfabrication process used to create microtextured biomaterial scaffolds (modified from Deutsch et al. [13]). (a) A layer of 1818 photoresist is spun onto a silicon wafer. (b) The wafer is exposed to UV light through a mask thereby selectively exposing the photoresist. (c) The wafer is developed washing away the exposed areas. (d) PDMS is cured on the microtextured wafer and then peeled away leaving (e) the PDMS with the pattern inverse to that on the wafer. (f) Representative optical image of 1818 master used to generate ~50 μm groove-width substrata. Scale bar represent 60 μm.

and extracellular matrix arrangement have demonstrated a correlation between structural organization and function. For example, collagen fibers oriented circumferentially using a magnetic field exhibit enhanced mechanical properties compared to unoriented fibers [6]. Three-dimensional microstructures [7–10] have been used to provide topographical cues to control cellular organization for a number of different cell types [11,12], but have not yet been used to organize and investigate VSMCs.

In this study, we fabricate polydimethylsiloxane (PDMS) tissue scaffolds with grooved relief structures of varying dimension to assess the effectiveness of these topographical cues to elicit VSMC orientations similar to that seen in the herringbone helical arrangement. We directly compare cellular orientation, morphology, and organization of FN on textured and nontextured substrata.

2. Materials and methods

2.1. Microfabrication of microtextured PDMS scaffolds

To create topographical features in scaffolds, photolithography and soft lithography are used to create multiple parallel grooves in polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning). Groove width is varied (~20, 50, and 80 μm), and groove depth and spacing between grooves are kept constant at 5 and 12 μm, respectively. These groove widths were chosen because most cell types are known to orient along fibers with diameters in a fairly narrow range of 5–50 μm [10].

Polymer scaffolds are fabricated as described previously by Deutsch et al. [13] (Fig. 1B). Briefly, a positive resist layer (Shipleys Microposit 1818) is spun onto a silicon wafer (University Wafers) and soft baked at 100 °C. The resist is patterned by UV exposure through a patterned chrome mask using a contact mask aligner (Karl Suss MJB-3). After exposure, the patterned silicon wafer is developed (Shipleys Microposit Developer diluted 1:1 with de-ionized water) and shaken continuously for approximately 35 s to remove the exposed areas of the 1818 layer from the wafer. This process creates an 1818 master that could be used several times to mold polymer tissue scaffolds. The pattern dimensions on the master are verified with a profilometer (Alpha-Step 500 KLA Tencor). Microtextured scaffolds are formed by pouring PDMS (10:1 prepolymer: curing agent) onto the master and curing for 2 h at 80 °C. Control nontextured PDMS scaffolds are created by curing PDMS onto the surface of an unmodified silicon wafer. Pattern transference from the 1818 master to the PDMS scaffold is verified by optical microscopy (Olympus BX60, 20X).

2.2. Cell culture on PDMS scaffolds for cell alignment and morphological studies

To test the effects of microtopography on cell response, human umbilical smooth muscle cells (HUVS-112D, American Type Culture Collection (ATCC), Manassas, VA) are cultured on microtextured and nontextured PDMS scaffolds. The cell media used for all experiments is 90% Ham's F-12K medium supplemented