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Influence of nanopatterns on endothelial cell adhesion: Enhanced cell retention under shear stress

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Abstract

In this study, nanopatterned crosslinked films of collagen Type I were seeded with human microvascular endothelial cells and tested for their suitability for vascular tissue engineering. Since the films will be rolled into tubes with concentric layers of collagen, nutrient transfer through the collagen films is quite crucial. Molecular diffusivity through the collagen films, cell viability, cell proliferation and cell retention following shear stress were studied. Cells were seeded onto linearly nanogrooved films (groove widths of 332.5, 500 and 650 nm), with the grooves aligned in the direction of flow. The nanopatterns did not affect cell proliferation or initial cell alignment; however, they significantly affected cell retention under fluid flow. While cell retention on unpatterned films was $35 \pm 10\%$, it was $75 \pm 4\%$ on 332.5 nm patterned films and even higher, $91 \pm 5\%$, on 650 nm patterned films. The films were found to have diffusion coefficients of *ca.* 10^{-6} cm^2 s^{-1} for O_2 and 4-acetaminophenol, which is comparable to that observed in natural tissues. This constitutes another positive asset of these films for consideration as a scaffold material for vascular tissue engineering.

Keywords: Shear stress; HMEC; Collagen; Nanopattern; Vascular tissue engineering

1. Introduction

Cardiovascular diseases are the leading cause of death in developed countries, and the most common cause is atherosclerosis, a chronic pathological process associated with a raised focal plaque within large vessel intima. Typically, this plaque consists of a lipid core surrounded by an extracellular matrix (ECM) and smooth muscle cells, and is covered by a fibrous cap [1]. In time, the lesion increases in size, restricts blood flow and eventually blocks the vessel. The solution offered at this stage is generally the removal of the diseased vessel segment and implantation of a homograft or a synthetic graft. Homografts are obviously the best choice, but their availability is limited and many factors, including multiple bypass surgery, repeat procedures and age of the patient, decrease their suitability [2]. An alternative to homografts are synthetic grafts [3–6]. Synthetic grafts generally have a lower level of patency because practically all materials are somewhat thrombogenic and clogging of implanted grafts is a common problem [7]. To solve this problem, many different approaches, including a variety of anti-thrombogenic coatings [6,8] and endothelial cell seeding [9,10], have been tried. These treatments have improved the performance of synthetic grafts to a certain extent but they could only be used to replace larger arteries (>6 mm inner diameter). A functional, tissue engineered vessel would be the ultimate solution to vascular reconstruction of smaller diameter vessels [11].

Patency of an engineered vascular graft depends on several parameters. Most importantly, it should have a continuous layer of endothelium to prevent thrombosis and consequent clogging [12]. There are many studies that show that endothelial cells adhere to and proliferate well on many different materials, including polyesters [13,14], and

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natural polymers like silk fibroin [15] and collagen [16]. However, a major concern is that similar cell behaviour is required under the hemodynamic conditions of the natural blood vessel [17]. More specifically, the endothelial cells need to be retained on a supporting scaffold during exposure to flow and the resultant physiological shear.

In an attempt to mimic a natural tissue environment and thereby to improve attachment and alignment, patterned surfaces have been used. There are studies showing that endothelial cells are aligned by micropatterns [16,18]; however, this is also reported to result in apoptosis due to cell anisotropy and resultant inadequate cell attachment to the surface [19,20]. Nanoscale topographies have also been tried, mainly to increase cell adhesion on synthetic surfaces. PLGA surfaces having nanoscale controlled roughness rather than patterns were tested for improved endothelial cell adhesion, but this resulted in lower cell adhesion than on unmodified planar surfaces [14]. On the other hand, nanoscale modification of alternative vascular graft materials, including polyurethane derivatives [21] and titanium [22], was also tried, and did succeed in increasing endothelial cell attachment and proliferation, apparently through increased surface area.

Another complication to obtaining a continuous layer of functional endothelial cells is biomaterial-induced toxicity. Kader and Yoder have recently shown that synthetic biomaterials cause endothelial cell death due to anoikis, a form of apoptosis caused by an inflammatory reaction due to an inappropriate interaction of cells with the surface [23]. They proposed that endothelial cells on synthetic biomaterials like polytetrafluoroethylene, polyethyleneteraphthalate, polyester and polyethylene produce increased amounts of superoxides and other reactive oxygen species which, in turn, induce a local inflammatory response and cell death. Collagen is a part of the natural ECM and has the required molecular motifs for proper cell attachment, making it a good candidate for a scaffold material [24]. When tested as a scaffold material for vascular tissue engineering, acid soluble type I collagen was found not to enhance blood coagulation, did not interfere with the viscoelasticity of the blood and did not induce excessive platelet adhesion or aggregation, and as such its suitability for vascular tissue engineering has been highlighted [25].

When a tissue-engineered vascular graft is designed, one of the requirements in addition to fluid and nutrient flow through the lumen is adequate graft permeability to oxygen, glucose and other metabolites. This is especially needed when an engineered vessel consists of a tubular scaffold with smooth muscle cells seeded on the periphery and endothelial cells seeded subluminally, where diffusion of nutrients through the scaffold for the endothelial cells would be a major issue. In previous studies, these collagen films were tested with vascular smooth muscle cells and developed into tubular form by crosslinking for vascular tissue engineering [26].

In this study collagen type I films nanopatterned with various dimensions were seeded with human microvascular endothelial cells (HMECs) and tested for vascular tissue engineering purposes. Small molecule diffusivity through the collagen films was measured with or without seeding with the endothelial cells. The molecules selected (oxygen and 4-acetaminophenol) were readily detectable electrochemically and their diffusion to a polarized electrode surface readily tracked [27]. Furthermore, cell viability, cell proliferation and cell adhesion strength during shear stress caused by medium laminar flow over the films was determined.

2. Materials and methods

2.1. Template preparation

Three different templates with nanochannels were created by X-ray interference lithography at the facilities of Bilkent University Physics Department (Ankara, Turkey). Briefly, first a photoresist (AZ 5214) was coated on a silicon wafer (500 nm thickness), exposed to a laser ($\lambda = 325$ nm) for 10 min and developed, then the patterns were transferred to silicon by using an epoxy replica. Subsequent poly(dimethylsiloxane) (PDMS) replicas of the modified silicon templates prepared by applying mixed PDMS and curing agent (in a 10:1 wt./wt. ratio; Sylgard 184 Elastomer Kit, Dow Corning, USA) and curing at 65 °C for 3 h for use in patterned collagen film preparation. The templates prepared had patterns of parallel channels with groove and ridge widths of 650 nm with 300 nm depth, 500 nm with 250 nm depth and 332.5 nm with 200 nm depth.

2.2. Collagen film preparation

Collagen films (1 cm^2) were prepared by solvent casting collagen solution on nanopatterned and unpatterned PDMS templates with overnight drying at room temperature (RT). Collagen type I was isolated from Sprague–Dawley rat tails and used at a concentration of 10 mg ml^{-1} solution (250 μl cm^{-2}, 0.5 M, in acetic acid). Films were stabilized by chemical crosslinking for 2 h at RT using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC, Pierce, USA) and *N*-hydroxysulfosuccinimide (NHS, Sigma Chemical Co., USA). The concentrations used were 170 mM EDC and 217 mM NHS in 50 mM NaH_2PO_4 solution (pH 5.5). After crosslinking, films were washed with 0.1 M Na_2HPO_4 (pH 9.1) for 1 h, with 1 M NaCl for 2 h and with 2 M NaCl for 1 day, each with a minimum of five solution changes [28]. Later, films were washed several times with distilled water and peeled off the surface with forceps after drying at RT. Films were examined after crosslinking using scanning electron microscopy (SEM) for pattern fidelity.

2.3. Cell culture studies

HMECs (CDC of USA, CDC Reference Number: E-036-91/0, passage 15) were cultured in MCDB 131 Medium (Gibco, USA) supplemented with 5% fetal calf serum (Gib-