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Overcoming foreign-body reaction through nanotopography: Biocompatibility and immunoisolation properties of a nanofibrous membrane

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ABSTRACT

Implantable immunoisolation membranes need to possess superior biocompatibility to prohibit the fibrotic deposition that would reduce the nutrient supply and impair the viability/function of the encapsulated cells. Here, electrospun membranes based on thermoplastic polyurethane (TPU) were fabricated to contain microfibers (PU-micro) or nanofibers (PU-nano). The two types of membranes were compared in terms of their interaction with macrophage cells and the host tissues. It was found that the fibrous membranes of different topographies possess distinct material properties: PU-nano caused minimal macrophage responses *in vitro* and *in vivo* and induced only mild foreign body reactions compared to PU-micro membranes. A flat macroencapsulation device was fabricated using PU-nano membranes and its immunoisolation function investigated in subcutaneous transplantation models. The nanofibrous device demonstrated the capability to effectively shield the allografts from the immune attack of the host. Nanotopography may confer biocompatibility to materials and nanofibrous materials warrant further study for development of "invisible" immunoisolation devices for cell transplantation.

1. Introduction

With rapid advancements in cell/stem cell technologies in recent years, there are great needs to develop biomaterial-based devices to aid cell transplantation in terms of maintaining cell viability and function in host. In particular, immunoisolation devices aim to provide solutions to protecting transplanted cells from the immune attack of the host. The concept typically involves a semipermeable material to carry out dual tasks: 1) prohibit the invasion of immune cells/cytokines into the device 2) allow exchange of nutrients across the artificial barrier so that the transplanted cells can function normally in the recipient's body [1]. The immunoisolation devices may find important applications in cell therapies, where the host's immune system imposes threats to transplanted cells, or vice versa, the transplants transmit potential risks (e.g. teratoma formation by embryonic stem cells) into the body [2]. Among different applications, designing devices to enable the transplantation of islet allo- or xeno-grafts to treat diabetes have been intensively investigated over the last four decades [3]. A few types of devices, e.g., alginate microcapsules and hollow fiber membranes encapsulating islets were shown to reverse hyperglycemic conditions in diabetic animal models [1,4,5].

Despite previous efforts, developing functional materials/devices facilitating cell transplantation and meeting all the requirements for clinical use remains a formidable challenge. One obstacle yet to be tackled is to search for semi-permeable materials having superior biocompatibility to combat tissue responses that are menaces to the encapsulated cells. It is thought that the long-term efficacy of an immunoisolation device would be affected by the foreign-body response (FBR), which is manifested in dense, fibrotic collagen capsules that could cut off the transportation of oxygen and nutrients into the device [1,6]. Synthesizing new materials or alternating the chemistry of current materials has been pursued for immunoisolation devices [7,8]. Another approach to

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modify or optimize the tissue interaction with materials is through designing appropriate topographical/structural characteristics of surfaces [9,10]. Elucidating the fundamental relationship between the surface topographical structure and material biocompatibility properties therefore has important implications for developing high-performance devices.

In this work, we aim to investigating the fundamental properties inherent to nanofibrous electrospun materials for use as semipermeable immunoisolation membrane. Nanofibrous structure mimics the topographical features of natural extracellular matrices (ECM). In particular, electrospinning allows fabrication of fibrous matrices with controllable structural characteristics including fiber diameter, pore size and thickness via facile modulation of processing parameters [11]. Electrospun fibrous materials thereby have attracted intensive interests in recent years for different biomedical applications including filtration [12] and tissue scaffolds [13,14]. Previous studies have shown that nanoscale fiber morphology may provide unique contact cues to modulate macrophage cells toward anti-inflammatory phenotypes *in vitro* [15,16]. However, it is unknown whether nanofibrous membranes could also demonstrate advantages in mediating cell and tissue responses *in vivo* and find applications in immunoisolation devices.

Here, experiments were carried out to understand the fundamental properties of fibrous materials dictated by fiber sizes. Our study suggests that electrospun nanofibers induced only minimal FBRs with indiscernible activation of macrophage cells compared to microfibrous membranes. Nanofibrous materials can therefore serve as a new category of semipermeable materials for use in cell transplantation and immunoisolation.

2. Materials and methods

2.1. Fabrication and characterization of electrospun membranes

CarboSil™ thermoplastic silicone polycarbonate urethane (TPU) solutions were prepared by dissolving TPU in a 3:2 (v/v) mixture of tetrahydrofunan (THF) and N, *N*-Dimethylformamide (DMF). The custom-built electrospinning device was equipped with a syringe pump (WZS 50-F6 , Zhejiang University Medical Instrument Co., Ltd, Hangzhou, China), a 10-mL syringe, a stainless steel blunt needle, a collector and a high voltage supply (EST-801A, ESD-China, Beijing, China). PU-nano and PU-micro membranes were fabricated with the parameters shown in Table 1.

The pore size of different membranes was measured via extrusion porosimetry (3H-2000 PB, Beishide Instrument, China). The bubble point was determined by the pressure needed to blow air through the liquid filled membrane. The range of pore sizes (D) was calculated using the Washburn equation: $D = \frac{-4\gamma\cos\theta}{P}$, where P is the differential pressure, γ the surface tension of the wetting liquid and θ the wetting angle. For a complete wetted membrane with all pores filled with the wetting liquid, cos θ is 1.

To observe the morphology of different fibers, membrane samples were gold sputter-coated and examined by the scanning electron microscope (SEM) (S-4800, Hitachi, Japan). ImageJ (NIH, Bethesda, USA) was applied to quantitatively characterize the fiber diameter. For each sample, 10 random fibers were selected and measured in each image, and a total of 10 images were counted.

To study the tensile strength of the electrospun fibers, the electrospun membrane was mounted on the Table-top Precision Testers (AGS-X, Shimadzu, Japan) and pulled at a constant strain rate of 5 mm/min until fracture. The Young's modulus was obtained by measuring the slope of the stress-train curve in the elastic region. Four samples were tested for each type of membrane.

To perform nanoindentation tests on the level of single fibers, electrospun fibers were collected on a smooth uniform glass slides for 15 s. The sample was then imaged and a single fiber for testing was located by the indenter. The quasi-static indentation tests was performed by using a TriboIndenter (Hysitron, Minneapolis, USA) with a standard 50 nm Berkovich diamond tip. The indentation loads, together with the corresponding displacement data, were used to calculate the indentation modulus. Five samples were tested for each type of membrane.

The protein adsorption on the electrospun fibers was measured by the BCA protein assay kit (Pierce, Rockford, USA). The fibers were cut into ~5 mm^2 pieces and the exact mass recorded. The samples were equilibrated in DPBS for 1.5 h before incubation with 10% FBS solutions for 24 h. The adsorbed protein was then dissolved in 1% SDS after gentle shake and then quantified. The data is reported as the average mass of adsorbed protein normalized over the membrane mass ($n = 5$).

2.2. Cell preparation and culture

Rat adipose derived mesenchymal stem cells (AD-MSCs) were obtained from the subcutaneous adipose tissue of male Sprague–Dawley rats (Vital River Laboratory, China) weighing 120 g according to a reported protocol [17]. AD-MSCs were cultured and passaged in the MEM Alpha supplemented with 10% (v/v) fetal bovine serum (FBS) and 0.4% penicillin/streptomycin (P/S). Freshly isolated primary rat hepatocytes were a kind gift from Dr. Hong-kui Deng, Peking University.

Pancreatic islets were isolated from 12-week-old male C57BL/6 mice. The bile duct was cannulated with a 27 G needle and the pancreas distended with 0.5 mg/mL cold Collagenase P (Roche Diagnostics, Germany) in Hanks' balanced salt solution (Gibco, USA). The perfused pancreases were then removed and digested in a 15-mL tube placed in a 37 °C incubator. Subsequent to digestion, islets were first purified on histopaque-density gradients (Sigma-Aldrich, WI, USA) and then handpicked under the microscope. The islets were cultured in RPMI-1640 medium with 10% FBS.

The RAW 264.7 macrophages and 3T3 fibroblasts were obtained from the Cell Culture Center of the Institute of Basic Medical Sciences (Beijing, PR China). The macrophages and fibroblasts were cultured and passaged in high-glucose DMEM supplemented with 10% FBS and 1% P/S. Luciferase expressing 4T1 mouse breast cancer cell lines (4T1-luc) were obtained from Perkin Elmer (Waltham, MA, USA). The cells were cultured and passaged in RPMI 1640 media containing 10% FBS and 1% P/S.

2.3. In vitro cytocompatibility of electrospun fibers

The electrospun fibers were cut into 13 mm disks and then sterilized with 70% ethanol. 10,000 3T3 fibroblasts were seeded on the disks and cultured for 2 days to evaluate the proliferation

Table 1
Electrospinning conditions for fabricating PU-nano and PU-micro membranes.

	Flow rate (mL/h)	Needle (G)	Applied voltage (kV)	Distance from collector (cm)	Solution concen. (%, w/v)
PU-nano	0.5	25	13	13	8
PU-micro	1	21	9	10	15