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Incorporation and selective removal of space-forming nanofibers to enhance the permeability of cytocompatible nanofiber membranes for better cell growth

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ABSTRACT

Nanofibrous biomaterials fabricated by electrospinning attract increasing attention. However, electrospun nanofibers are stacked tightly, thus restricting cell infiltration and nutrient supply. To overcome this drawback, we developed three types of cytocompatible nanofiber membranes, namely (1) electrospun chitosan/gelatin/PEO (CGP) composite membrane, (2) co-electrospun CGP/E membrane containing CGP and Eudragit (E) nanofibers, (3) CGP (E removed) membrane which was obtained by selective removal of E nanofibers from CGP/E membrane. The permeability of albumin through CGP, CGP/E, and CGP (E removed) nanofiber membranes increased from 1.50×10^{-11} to 6.19×10^{-11} and further to 15.3×10^{-11} m^2/s, respectively. Hence, incorporating large-diameter (space-forming) E nanofibers with CGP nanofibers enhanced the permeability of CGP/E membrane. Moreover, selectively removing E nanofibers from CGP/E membrane further enlarged pore size and created more permeable CGP (E removed) membrane. Membrane strength and stability were reinforced by crosslinking with glutaraldehyde for 2.5 h. To verify our approach, stem cells (KP-hMSCs) were covered by CGP (E removed) or CGP membrane and cultured for 7 days. The cell density underneath CGP (E removed) membrane was about 1.7 times of that underneath CGP membrane, indicating that improved cell growth (proliferation) occurred under CGP (E removed) membrane. Therefore, our approach to create more permeable nanofiber membranes for better cell growth is successful and can be utilized for fabricating various nanofibrous biomaterials.

1. Introduction

Fabrication of nanofiber membranes by electrospinning attracts increasing attention since ultra-fine nanofibers have large specific surface area [1–4]. Further, nanofibers are structurally similar to the extracellular matrix (ECM) and thereby electrospun nanofiber membranes have great potential in biomaterials applications [5–7]. However, the electrospun nanofibers are typically stacked tightly, thus restricting cell infiltration and diffusion of nutrients or wastes through these nanofibers in a 3-D cell culture system [8].

To deal with this problem, a study focusing on varying the fiber diameter and packing density to control the porosity of fibrous constructs revealed that low packing density led to better cell proliferation and infiltration [8]. Further, another work using removable sacrificial fibers to increase void space in nanofibrous composites also showed success in accelerating cell infiltration and subsequent tissue formation [9]. However, the permeability values of molecules through these fibrous constructs were not determined. In this research, we tried to incorporate large-diameter (space-forming) nanofibers with small-diameter nanofibers to enhance mass transfer within the resulting nanofiber membrane. We also tried to selectively dissolve the large-diameter (space-forming) nanofibers to further enhance the mass transfer in the membrane (*i.e.*, to create more permeable nanofiber membranes). To confirm the effectiveness of our approach, the permeability values of albumin through various nanofiber membranes were measured and compared.

For fabricating cytocompatible electrospun nanofiber membranes, we chose chitosan, gelatin, and polyethylene oxide (PEO) to prepare a chitosan/gelatin/PEO (CGP) mixed solution and used

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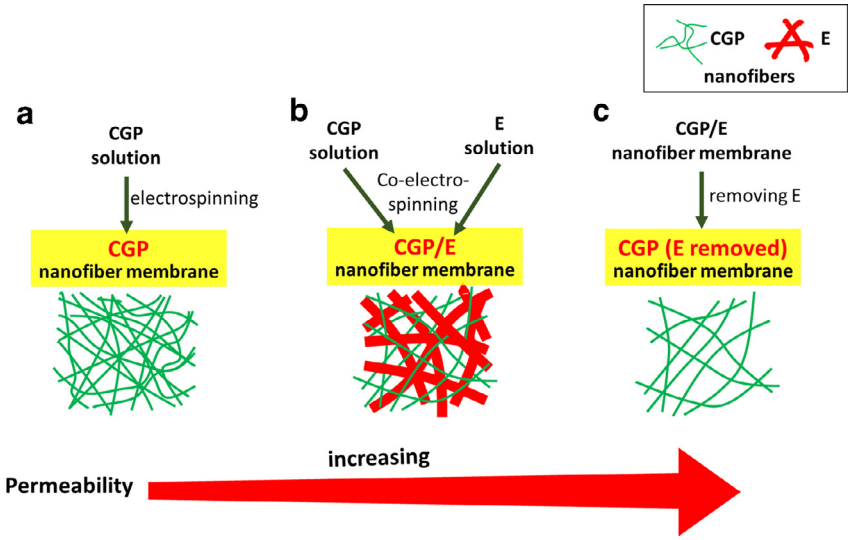

Fig. 1. Preparation of CGP, CGP/E, CGP (E removed) nanofiber membranes with increasing permeability: (a) CGP membrane was fabricated by single-needle electrospinning, (b) CGP/E membrane was fabricated by dual-needle co-electrospinning, (c) CGP (E removed) membrane was obtained by immersing CGP/E membrane in ethanol to dissolve and thus selectively remove E nanofibers.

it to produce CGP nanofiber membranes by electrospinning. The reasons for choosing these materials are as follows. Chitosan is an abundant natural polysaccharide composed of glucosamine and N-acetyl glucosamine [10,11]. It is non-toxic and cytocompatible, hence being commonly used as a biomaterial, *e.g.*, in the form of electrospun nanofibers [12–15]. Gelatin is derived from partial hydrolysis of collagen and is widely used to improve the cytocompatibility of a biomaterial for various biomedical applications [16,17]. PEO of ultra-high molecular weight was used to improve the electrospinnability of chitosan/gelatin mixed solution [18]. To enhance mass transfer within a nanofiber membrane, Eudragit® S-100, a copolymer of methacrylic acid and methyl methacrylate which is ethanol-soluble but water-insoluble, was used to form large-diameter (space-forming) Eudragit (E) nanofibers during co-electrospinning with small-diameter CGP nanofibers.

In this research, three types of cytocompatible nanofiber membranes, namely CGP, CGP/E, and CGP (E removed) nanofiber membranes, were prepared (Fig. 1). CGP membrane was fabricated by single-needle electrospinning of CGP solution; CGP/E membrane was made by dual-needle co-electrospinning of CGP and E solutions; CGP (E removed) membrane was obtained by immersing crosslinked CGP/E membrane in ethanol to dissolve and thus remove E nanofibers. In this way, the three nanofiber membranes with increasing permeability were created (Fig. 1). To examine whether our approach to create more permeable nanofiber membranes is effective, we cultured human mesenchymal stem cells (KP-hMSCs) underneath various nanofiber membranes (to mimic microenvironment within the membrane) and found that cell growth underneath CGP (E removed) membrane was significantly improved.

2. Materials and methods

2.1. Materials

Chitosan (M.W. = 300 kDa, degree of deacetylation = 90%) was purchased from Kiotek (Taipei, Taiwan). Gelatin (G9391) and PEO (M.W. = 5,000 kDa) were purchased from Sigma-Aldrich (St. Louis, USA). Eudragit® S-100 (M.W. = 125 kDa) was supplied by Evonik Industries AG, (Darmstadt, Germany). All other solvents and chemicals were reagent grade.

2.2. Preparation of solutions

For preparing chitosan/gelatin/PEO (C/G/P) mixed electrospinning solution, gelatin and PEO powders were first dissolved in water. Chitosan powders were then added and well-suspended in the solution, followed by the addition of acetic acid solution as a solvent to dissolve chitosan powders. Three CGP mixed solutions of different total concentrations (3.3, 5.5, and 10.5 wt%) were prepared and their compositions were shown in the left part of Table 1. For preparing Eudragit® (E) electrospinning solutions (5 and 10 wt%), Eudragit® powders were dissolved in 95% (v/v) ethanol.

2.3. Properties of solutions

The viscosity, conductivity and surface tension of various electrospinning solutions were measured using a viscometer (model DV-II+, Brookfield, USA), a conductivity meter (model COND6 plus, Eutech, Singapore) and a tensiometer (model 514-B, Itoh Seisakusho, Japan), respectively.

2.4. Preparation of CGP, CGP/E, and CGP (E removed) nanofiber membranes

The schematic illustration of the preparation of CGP, CGP/E, and CGP (E removed) nanofiber membranes with increasing permeability is shown in Fig. 1. Electrospinning was carried out in an electrospinning unit (Jyi-Goang Enterprise, Taipei, Taiwan) with CGP solution or E solution loaded in a 3 ml syringe (with 20 G needle) and fed at a flow rate of 1.2 ml/h by a syringe pump. The distance from electrospinning needle to collector was 15 cm with the applied voltage of 25 kV, environmental temperature of 32 °C and relative humidity of 40–60%. CGP membrane was fabricated by single-needle electrospinning of CGP solution (Fig. 1a). CGP/E membrane was made by dual-needle co-electrospinning of CGP and E solutions (Fig. 1b). CGP (E removed) membrane was obtained by immersing crosslinked CGP/E membrane in ethanol to dissolve and thus remove E nanofibers (Fig. 1c).

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