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Mild immobilization of diverse macromolecular bioactive agents onto multifunctional fibrous membranes prepared by coaxial electrospinning

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Abstract

Coaxial electrospinning was proved to be a facile method to produce multifunctional fibrous matrices which could essentially emulate certain features of native extracellular matrix. In order to further confer capability of immobilizing diverse macromolecular bioactive agents to the fibers, composite membranes composed of cationized gelatin-coated polycaprolactone (PCL) fibers were prepared by coaxial electrospinning. Gelatin was cationized by derivation with *N*,*N*-dimethylethylenediamine. The cationized gelatin (CG) was used as a shell material for constructing a core–shell fibrous membrane. PCL formed the core section of the core–shell fibers thereby improving the mechanical properties of nanofibrous CG hydrogel. The outer CG layer was crosslinked by exposing the membranes in glutaraldehyde vapor. The adsorption behaviors of FITC-labeled bovine serum albumin (FITC–BSA) or FITC–heparin onto the fibers were investigated. The core–shell fibers could effectively immobilize the two types of agents under mild conditions. The adsorption amount could reach about 12 μg of BSA per mg of membrane and 23 μg mg^{-1} for heparin. Furthermore, vascular endothelial growth factor (VEGF) could be conveniently impregnated into the fibers through specific interactions with the adsorbed heparin in the outer CG layer. Sustained release of bioactive VEGF could be achieved for more than 15 days.

Keywords: Electrospinning; Core–shell; Gelatin; Heparin; Growth factor

1. Introduction

The native extracellular matrix (ECM) is a three-dimensional network of biomacromolecules, and serves not only as a structural scaffold but also as an environment directing the actions of tissues and cells [1]. The basic structure of ECM is defined by a nanofibrous collagen scaffold. Adhesive glycoproteins, growth factors (GFs) and proteoglycans adhere to the collagen scaffold and interact with the cells in or adjacent to the matrix [2]. Designing ECM-mimicking artificial matrices or scaffolds has always been a key issue in the field of tissue engineering [3]. Due to the ECM-like topography, nanofibrous scaffolds have been extensively studied in recent years [4]. Various methods for preparing nanofibrous scaffolds have been explored. These include electrospinning, phase separation and self-assembly. Of these, electrospinning is a promising technique suitable for producing interconnected and highly porous fibrous scaffolds [5]. Up to now, numerous biodegradable polymers, such as poly(lactide-co-glycolide) (PLGA) [6], poly(ε-caprolactone) (PCL) [7], chitosan [8], collagen [9] and gelatin [10], have been successfully electrospun into non-woven porous matrices for tissue engineering.

Gelatin is a proteinaceous material prepared by hydrolytic degradation of naturally occurring collagen. Because of its biodegradability, biocompatibility and excellent cell affinity, it has been given a great deal of attention in the field

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of tissue engineering [11]. The electrospun gelatin membranes have been successfully prepared in various solvents, such as trifluoroethanol (TFE) [10], 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) [12] and formic acid [13]. The nanofibrous scaffolds of gelatin mimic not only the topography but also chemical composition of ECM, and could support the growth of various cells [12,14]. However, it was found that the electrospun gelatin membrane was subjected to a significant decrease in its porosity and pore size because of crosslinking [15,16]. An implication of the reduced pore size of the scaffolds is the formation of barriers for preventing cell migration from the exterior of the scaffold matrix to its interior, thereby limiting their potential for tissue engineering and other related applications. For circumventing the above limitations, core–shell fibrous scaffolds composed of gelatin-coated PCL were fabricated by coaxial electrospinning [16]. The presence of PCL in the core section of the core–shell fibers significantly enhanced both the morphological stability and mechanical strength of the fibrous membranes.

Macromolecular bioactive agents, such as anionic polysaccharides (i.e., heparin, hyaluronic acid and DNA) and GFs play important roles in harnessing and controlling cellular functions in tissue regeneration [17]. Ideal tissue engineering scaffolds should not only mimic the topography and compositions of ECM, but also be integrated with macromolecular bioactive agents in order to finely modulate the cell migration, proliferation and differentiation [4,18–20]. For this purpose, cationized gelatin (CG) was used as a shell material for fabricating core–shell fibrous membranes in this study. The adsorption behaviors of bovine serum albumin (BSA) and heparin onto the outer CG hydrogel were studied. BSA was selected as a model protein with isoelectric point (IEP) below pH 7.4 and heparin as a model of anionic polysaccharides. Since numerous GFs, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), could bind strongly to heparin [21], it would also be convenient for them to be immobilized onto the surface of the core–shell fibers through the specific interactions with the pre-adsorbed heparin. In addition, the presence of heparin in the outer CG hydrogel could effectively stabilize the immobilized GFs during adsorption and release processes [21,22].

2. Materials and methods

2.1. Materials

PCL (*MW* 73,000) was synthesized by ring opening polymerization of ε-caprolactone at 140 °C using stannous 2-ethyl hexanoate as a catalyst. The molar mass of the product was determined by GPC using THF as a solvent. Gelatin (type A), bicinchoninic acid (BCA), fluoresceinisothiocynate (FITC), bovine serum albumin (BSA) and heparin were purchased from Sigma–Aldrich (Milwaukee, WI, USA). VEGF and ELISA kit for VEGF were supplied by PeproTech (Rocky Hill, NJ, USA). Trifluoroethanol (TFE) was obtained from Xinyuan Chemical Corporation (Shandong, China) and used without further purification. Glutaraldehyde (GA, 25% aqueous solution) and *N*,*N*-dimethylethylenediamine (DMED) were supplied by Acros Organics (Geel, Belgium). Double-distilled water was used.

2.2. Characterization

Surface morphologies of the electrospun scaffolds were observed by scanning electron microscopy (SEM, JEOL JSM-5300). Samples for SEM were dried under vacuum, mounted on metal stubs, and sputter-coated with gold–palladium for 30–60 s. The average diameters and distribution of the fibers were analyzed with the software Image-Pro Plus (Media Cybernetics, Inc.). Fiber spacing (D_s) was calculated according to Eq. (1), where N_o is the fiber number within a defined region and A is the region area [23]. The fiber number was counted with the software Image-Pro Plus. The cross-section of the fibers was exposed by freeze fracturing and observed with SEM. Tensile tests of the electrospun membranes were carried out on a Zwicki Z2.5/TH1S Universal Mechanical Testing Machine (Zwick, Ulm, Germany) equipped with GTM load cell. The 1×4 cm rectangular specimen of approximately 200–250 μm thickness was vertically mounted onto the two mechanically gripping units of the machine at their ends, leaving a 4 cm gauge length for mechanical loading. The tensile rate was set at 0.4 mm min^{-1}.

$$D_s = \frac{0.5}{\sqrt{N_o/A}} \quad (1)$$

2.3. Synthesis of cationized gelatin (CG) [24]

Five grams of gelatin was dissolved in 250 ml of 1/15 M KH_2PO_4 buffer solution (pH 5.0). EDC (0.89 g) and a certain amount of DMED were added. Immediately after that, the pH of the reaction mixture was adjusted to 5.0. The reaction mixture was magnetically stirred at room temperature for 24 h, and then dialyzed against distilled water with cellulose membrane (*MW* cut-off 3500) for 2 days. The cationized gelatin was obtained by freeze drying of the dialyzed solution.

2.4. Colloid titration

The substitution degree of DMED in CG was estimated by colloid titration. In brief, 25 ml of aqueous CG solution (1 mg ml^{-1}) was titrated with aqueous heparin solution (1 mg ml^{-1}) until approaching the titration end point (i.e., maximum turbidity). Prior to titration, the pH of both CG and heparin solutions was adjusted to 7.4. The turbidity was monitored with a UV–vis spectrophotometer at 420 nm and reported as 100-T%, which was linearly proportional to the true turbidity for $T > 0.9$. The substitution degree of DMED in CG (DS) was calculated according to Eq. (2), where M_c^{CG} and M_c^{gel} are the molar contents of cations in 1 g of CG and gelatin, respectively, and M_a^{gel} is the

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