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Improving cytoactive of endothelial cell by introducing fibronectin to the surface of poly L-Lactic acid fiber mats via dopamine

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ABSTRACT

A simple but straightforward approach was reported to prepare fiber mats modified with fibronectin (Fn) protein for endothelial cells activity study. Based on the self-polymerization and strong adhesion feature of dopamine, poly L-Lactic acid (PLLA) fibers mat was modified via simply immersing them into dopamine solution for 16 h. Subsequently, Fn was immobilized onto the fiber mats surface by the coupling reactive polydopamine (PDA) layer and Fn. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) were used to determine the chemical compositions of fiber mats surface, which confirmed the successful immobilization of PDA and Fn molecules on the fiber surface. Scanning electronic microscopy (SEM) was used to observe the surface morphology changes after modification with PDA and Fn. The data of water contact angle showed that the hydrophilicity of the fiber mats was improved after surface modification. The data of in vitro cell culture proved that the PDA and Fn modified surface significantly enhanced the adhesion, proliferation and cell activity of endothelial cells on the fiber mats. And the release of tumor necrosis factor-α (TNF-α) by endothelial cells on the modified surface was suppressed compared to that on culture plate and PLLA film at 2 and 4 days, while the secretion of interleukin-1β (IL-1β) was increased compared to that on culture plate and PLLA film at 2 days.

1. Introduction

Tissue engineering has appeared to be the most promising technology to improve, repair, or replace damage tissue or organ function [1,2]. Significant challenges to this technology include the design and fabrication of a biodegradable 3D cell scaffolds which could promote cell adhesion, growth, proliferation, and differentiation. In native tissues, the diameter of extracellular matrix (ECM) structural proteins ranges from 50 to 500 nm. In order to create analogue scaffolds, nanotechnology can be employed at this scale. Recent advances in nanotechnology had led to a variety of approaches for the development of engineered ECM analogues. Electrospinning technique had been proven successful in routinely creating nanofibrous tissue engineering structures [3–6].

The electrospun fibers had large surface to volume ratio, increased porosity and good mechanical properties. Compared to traditional methods, electrospinning was much more suitable for the creation of scaffolds with three dimensional structures similar to ECM [7,8]. Electrospinning had been widely used for cell culture studies and tissue engineering scaffolds design [9,10]. In order to improve the biocompatibility and hydrophilicity of original electrospun fibers, surface modification was often used. The fiber mats modified with hydrophilic group could improve the hydrophilic and cell compatibility significantly [11]. And the mats modified with biologically active molecules had proved to be an effective method to improve their blood compatibility, also could increase the cell adhesion and proliferation properties significantly [12,13].

Recent bionics research found that 3,4-dihydroxyphenethylamine (dopamine) was able to self-polymerize in aqueous solution and form strongly attachment to a wide range of substrates such as rocks, metals, polymers and wood [14]. Compared to other surface modification technology such as grafting [15], entrapment [16], plasma treatment [17,18] and self-assembly [19], polydopamine (PDA) modification was more convenient and easily to achieve. Based on the strong adhesion behavior and reactivity of PDA layer, a facile and effective approach for biomaterials surface modification had been developed [20]. By simple immersion operation, a PDA layer could adhere on the material surfaces successfully. The polar groups on PDA coating, such as hydroxyl and amine groups, endowed the substrates with improved hydrophilicity and anti-fouling ability. More importantly, PDA layer containing reactive groups provided a versatile platform for further surface functionalization [21]. Fibronectin (Fn) was known to regulate cell growth, differentiation and spreading, In fact, biomaterial surfaces coating with Fn had been shown to enhance the formation of focal adhesions by osteoblasts in vitro [22], and led better cell spreading and cytoskeleton organization compared to non-coated surfaces [23–25].

In this paper, we proposed a facile method to generate biomolecular on poly L-Lactic acid (PLLA) electrospun fibers mats by soak with

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dopamine and Fn solutions. The fiber mats was first produced by electrospinning PLLA solution. The sample was then immersion in dopamine solution to form a thin layer on the surface of fiber mats. Fn was grafted on the material through PDA layer on fiber mats surface. After optimization, endothelial cells were cultured on the Fn modified fiber mats. Cell activity and the inflammatory factor expression of endothelial cells on these mats were then analyzed and recorded, showing application potential of the hybrid system for tissue engineering purposes.

2. Materials and methods

2.1. Materials

PLLA (100,000 MW) was received from Daigang Biotech (Jinan, China). Dichloromethane (DCM) was purchased from Damao (Tianjing, China) and *N*,*N*-Dimethyl formamide (DMF) was obtained from Fuyu (Tianjing, China). Tris(hydroxymethyl) aminomethane (Tris) was obtained from Sijia (Guangzhou, China). Fn and dopamine were received from Sigma Aldrich. Tumor necrosis factor-α (TNF-α) assay kit and interleukin-1β (IL-1β) assay kit were purchased from Shanghai Yisaike Bio-Technique Co. Ltd.

2.2. Preparation of PLLA fiber mats

PLLA (7 wt%) was dissolving in DCM/DMF (4:1 v/v) mixed solution for electrospinning. The solutions were electrospun from a 10 mL syringe with a blunt-ended needle diameter of 0.4 mm under 18 kV high voltage and 16 cm receiver distance. The injection rate was set as 1.0 mL/h. The fibers were collected using a rotating mandrel at a speed of 200 rpm. The samples were dried in vacuum at 40 °C for 12 h to eliminate the residual organic solvent.

2.3. Surface modification of PLLA fiber mats

Dopamine solution with a concentration of 2.0 mg/mL was prepared by adding dopamine in Tris-HCl buffer solution (pH = 8.5). The PLLA fiber mat was ultrasonically wetting with ethanol, and followed washing with deionized water prior to use. Then, the mat was immersed into dopamine solution in contact at room temperature for 16 h. After the reaction, the fiber mats were taken out and washed with deionized water thoroughly. After dried in a vacuum oven at 40 °C for 24 h, the modified PLLA fiber mat with PDA coating (PLLA-PDA) was obtained.

Fn solution with a concentration of 100 μg/mL was prepared by adding Fn into PBS solution, and then PLLA-PDA fiber mat was immersed in Fn solution at room temperature for 60 min. After the reaction, the fiber mats was taken out and rinsed with deionized water for three times, herein after referred to as PLLA-PDA-Fn. The process of preparation of modified PLLA fiber mats also was illustrated in Fig. 1.

2.4. Characterization of fiber mats

The morphologies of the PLLA fiber mats before and after modification were observed using scanning electron microscopy (SEM) (XL30 FESEM FEG, PHILIPS) at accelerating voltage of 20 kV. Before observation, the fiber samples were coated with a gold layer.

The hydrophilic properties of the surfaces of PLLA fiber mats before and after modification were characterized by contact angle measurement, using a contact angle goniometer (DKA100, Kruse) equipped with video capture. A piece of 1×3 cm^2 mat was attached on a glass slide. For the changes in water contact angle measurement, a drop of ultrapure water was dropped on the airside surface of the fiber mats, and the contact angle was measured after 2 s. The experiments were repeated three times, and the data were expressed as mean ± SD.

The FTIR data was collected on ATR-FTIR (Bruker Equinox 55, Germany) to analysis the surface chemical structures of PLLA, PLLA-PDA and PLLA-PDA-Fn, respectively. The spectra were measured in a wave number range from 4000 to 500 cm^{-1}.

The surface composition of the pristine PLLA, PLLA-PDA and PLLA-PDA-Fn were characterized by X-ray photoelectron spectroscopy (XPS) (ESCALAB 250, Thermo-VG Scientific) with a monochromated Al/Ka (hv = 1486.6 eV) as X-ray source. Surface spectra were collected over a range of 0–1200 eV and higher solution spectra of C1s, O1s and N1s regions were provided. The concentration of the different samples was determined by peak-area ratios. The data analysis was carried out with the Multipak software provided by manufacturers.

2.5. Morphologies of endothelial cells on PLLA fiber mats

To evaluate the morphologies of endothelial cells on PLLA fiber mats before and after modification, the fiber mats were cut into appropriate size and put in 24-well culture plate, selected the blank well as a control.

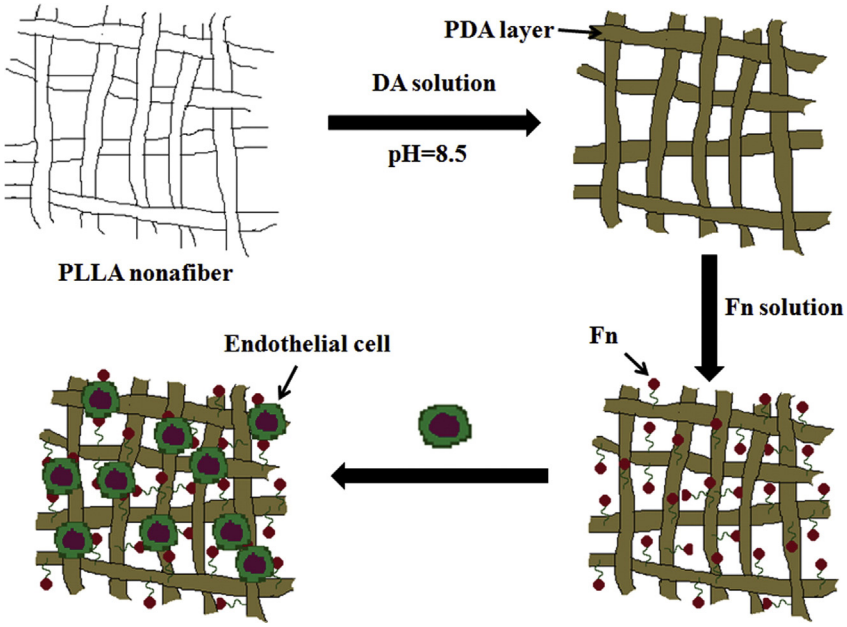

Fig. 1. Schematic illustration of the preparation process for PLLA fiber mats.