Study on Electrospun Poly(lactic acid)/Silk Fibroin-Gelatin Composite Nanofibrous Scaffold for Tissue Engineering

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Abstract: In this study, poly(lactic acid) (PLA) and silk fibroin(SF)-gelatin were sequentially electrospun on the collector to prepare the PLA/SF-gelatin composite fiber membranes. Scanning electronic microscope (SEM) was used to observe the morphology of the membranes. The microstructure of the fiber membranes before and after treatment with methanol was characterized using Fourier transform infrared (FTIR) and differential thermal analysis (DTA), and then the effects of the microstructure change on the mechanical properties, dissolution rate and shrinkage were evaluated. Furthermore, SEM and MTT assays were preformed to assess the proliferation and adhesion of 3T3 mouse fibroblasts cultured on the PLA/SF-gelatin scaffolds. After chemical treatment with methanol, the findings suggested the microstructure of PLA fiber did not change obviously, whereas the β-sheets of SF and α-helix structures of gelatin in SF-gelatin blend nanofibers increased, consequently the diameter of electrospun PLA/SF- gelatin membranes, the dissolution rate declined by 64%, the strain at break electrospun PLA/SF-gelatin scaffolds, the shrinkage of the PLA/SF –gelatin composite scaffolds caused by chemical treatment decreased by 40%. The cells adhered on to the surface of blend layer fibers and formed a confluent monolayer after 12 days cultivation. The good mechanical properties and dimensional stability of the chemically treated PLA/SF -gelatin scaffolds, combined with the ability to support cell growth in vitro, suggested tremendous potential application in tissue engineering.

Keywords: Electrospinning, nanofibers, PLA, silk fibroin, gelatin, scaffold.

1. Introduction

SF and gelatin are both natural biopolymers and have good biocompatibility and biodegradation, containing ligand (Arg-Gly-Asp, which can be recognized by integrin receptor in the cell membrane) that can promote cell differentiation, proliferation and adhesion. Kim [1] and jonathan [2] fabricated SF nanofiber membranes. Min [3] and Jin [4] further cultured human keratinocytes, fibroblasts and human bone marrow stromal cells on SF Nanofibers and investigated the feasibility for the wound dressing and cell scaffolds. Zheng-Ming Huang [5] investigated the Electrospinning of gelatin. Zhang [6] further performed the cross-linking of the electrospun gelatin nanofibers to improve their water-resistant ability and thermal-mechanical performance.

Although SF and gelatin can be electrospun into nanofibers and have the capacity of mimicking native extracellular matrix, they were generally water soluble and mechanically weak. To overcome these limitations, cross-linking was considered to be the most effective method because it could change molecular conformation [4,7-9]. However, these methods often resulted in the formation of brittle matrices due to high content of crystalline structures and the dimensional stability became poor.

Synthetic biodegradable polymers such as PLA and poly(lactide-co-glycolide) (PLGA) have recently become the promising scaffold materials. Yang [10] produced an aligned PLA nanofibrous scaffold for the study of neurite outgrowth and differentiation of neural stem cells seeded onto scaffolds. However, these synthetic polymers which have poor hydrophilic properties are well known in lacking bioactivity and cell affinity, for example, PCL did not promote the proliferation and adhesion of smooth muscle cells [11].

To achieve a strong and pliable scaffold that was low scaffold that has low dissolvability and excellent biological properties, SF and gelatin were electrospun and deposited on the PLA fibrous layer firstly formed by multi-electrospinning to construct bi-layered scaffolds. PLA is responsible for the resistance to rupture and shrinkage and confers the necessary elasticity, SF-gelatin and nanofiber layer support cell growth. The microstructure of the PLA/SF-gelatin nanofiber scaffold was examined before and after the treatment with methanol, and then the effects of the

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microstructure change on the mechanical properties, shrinkage and dissolution rate were investigated. MTT assay and SEM evaluated the adhesion and proliferation of mouse fibroblasts seeded on fibrous scaffolds.

2. Experimental section

2.1 Preparation of the composite PLA/SF-gelatin membranes

The air dried SF membranes, or a mixture of the air-dried SF membranes and gelatin with the weight ratio of 70:30, 50:50, were dissolved in 98%(w/w) formic acids yielding a 13%(w/w) spinning solution. PLA were dissolved in chloroform and acetone mixed solvent volume ratio of 2:1 to obtain a concentrated of 5%.

PLA Electrospinning parameters were as follows: voltage 25kv, flow rate 0.1 ml/h, distance between nozzle and collector was kept 15cm; for SF and SF-gelatin blend nanofiberes with different ratios were deposited onto the as-spun PLA fibrous membranes that Electrospinning, pure PLA and SF-gelatin nanofiber membranes were also fabricated according to the above parameters. These membranes were immersed in 100% methanol for 10min and dried for 24h at room temperature to form the chemically treated membranes

2.2 Morphology

SEM (Hitachi S-570, Japan) was used to observe the morphology of the scaffolds. Magnification for the PLA layer was 1000 times and the others were 10,000 times.

2.3 Characterization of microstructure

FTIR spectra were obtained by using a series spectrometer (Nicolet 570, PE Co. USA) in the spectral region of 4000-400cm^{-1}; DTA curves were obtained through a Thermal Analysis Instrument (Diamond 5700, PE Co. USA) at heating rate of 10℃/min, scan range of 40-400℃ and nitrogen gas flow rate of 120ml/min

2.4 Dissolvability and shrinkage test

The membranes were immersed in water with the bath ratio of 1:100(w/w) at 37±1℃ for 1h, then dried at 50℃ dissolution rates were calculated according the following formula:

$$\text{Dissolution rate} = \left(\frac{W_0 - W}{W_0} \right) \times 100\ \% \quad (1)$$

Where W_0 and W were the weights of the scaffolds before and after dissolution respectively.

As-electrospun membranes of PLA, SF-gelatin and PLA/SF-gelatin were cut into a square shape with dimensions of 2x2cm for shrinkage test, three samples in each group. The sizes and chemically treated membranes were measured and compared with initial dimensions. The shrinkage percentage was defined as the rate of the surface dimensional change of the recovered membranes.

2.5 Mechanical property

The tensile testing was performed by using an Instron tester (Model 3365). The length of samples was 30mm and the width was 6mm. The cross-head speed was20mm/min, five samples in each group. The mechanical property was characterized according to the following formula:

$$\text{Breaking tenacity} = \frac{\text{breakingstrength}(N)}{\text{thickness(mm)} \times \text{width(mm)}} \quad (2)$$

$$\text{Strain at break}\ (\%) = \left(\frac{L1 - L_0}{L_0} \right) \times 100 \quad (3)$$

Where L_0 was the length of sample, L_1 was the length at break.

2.6 Cell structure

3T3 mouse fibroblasts (Chinese Academic of Science, Shanghai, China) were cultured in DMEM containing 10% fetal bovine serum at 37℃ with 5% CO the medium was changed three times a week. After reaching about 80% confluence, the cells were detached by 0.25% trypsin. After treatment, the scaffolds were cut out and sterilized by γ ray and placed in 24-well plates. 3T3 mouse fibroblasts were cultured at a density of 5x10^4 per well in four different types of materials: (1) negative control well with no material; (2) PLA/SF; (3); PLA/SF-gelatin (50:50); (4) PLA/SF-gelatin (70:30) in particular, the cells were seeded on the SF or SF-gelatin layer of the composite membranes.