Composites: Part A 91 (2016) 20–29

Contents lists available at ScienceDirect

Composites: Part A

journal homepage: www.elsevier.com/locate/compositesa

Investigation of microporous composite scaffolds fabricated by embedding sacrificial polyethylene glycol microspheres in nanofibrous membrane

Junjiu Gao [a,b], Jiang Zhu [a,b], Jingjing Luo [a,c], Jie Xiong [a,b,*]

[a] College of Materials and Textiles, Zhejiang Sci-Tech University, Hangzhou 310018, China
[b] Key Laboratory of Advanced Textile Materials and Manufacturing Technology of Ministry of Education, Zhejiang Sci-Tech University, Hangzhou 310018, China
[c] College of Life Science, Zhejiang Sci-Tech University, Hangzhou 310018, China

ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form 18 September 2016
Accepted 19 September 2016
Available online 20 September 2016

Keywords:
A. Nano-structures
A. Polymer-matrix composites (PMCs)
B. Porosity
E. Electrospinning

ABSTRACT

The application of electrospun nanofibrous scaffolds in tissue engineering is limited due to their small pore size which leads to the failure of cell infiltration into the scaffolds. Microporous silk fibroin (SF)/gelatin composite scaffolds with biological properties could be fabricated to mimic tissue structure for tissue engineering applications. This study aimed to present a simple method to fabricate the composite scaffolds which is based on the simultaneously electrospraying of microspheres and electrospinning of nanofibers. Different contents of polyethylene glycol (PEG) microspheres were embedded into the SF/gelatin nanofibrous membranes by varying the content of PEG microsphere. Furthermore, microspheres can be selectively leached, obtaining the single SF/gelatin nanofibrous scaffolds and retaining the hierarchical organization of the porous nanofibers. In conclusion, the multilevel microporous and nanofibrous composite scaffolds can be obtained by varying pump flow rate of electrospraying.

1. Introduction

Despite the fascinating advances in the field of tissue engineering, the fabrication of hierarchically structured composite scaffold is still restrained by the inability to replicate complex tissue architecture by using traditional fabricating methods. Many of the current researches have focused on the potential of electrospun fibrous scaffolds for tissue-engineering applications because of their structural similarity to natural extracellular matrix (ECM) using biodegradable and biocompatible natural or synthetic polymers [1–3]. Silk fibroin (SF), a natural fibrous protein with high mechanical strength and good biological compatibility, is widely used in the biomedical field [4–7]. However, slow degradation rate of SF limited its use in clinical applications. Gelatin has been widely used to develop wound dressings due to the good compatibility and fast degradation *in vivo* [8,9]. Therefore, nanofibrous scaffolds prepared by SF and gelatin could mimic the ECM in terms of structure and chemical composition with controllable degradation rate [9–11]. However, the drawbacks of small pore size of electrospun nanofibrous scaffolds have limited the applications in three-dimensional culturing [12]. It's difficult for cells to migrate and infiltrate within the dense structure due to the roughly small pore size in the range of 200–1000 nm, since the cellular diameter is normally from 5 μm to a few tens of microns [13,14]. Therefore, it is significant to increase porosity and pore size of nanofibrous membranes for the application in tissue engineering.

Tremendous efforts have been made to develop three dimensional nanofibrous scaffolds using various techniques including sacrificial salt [15], direct laser machining [16], and electrospinning with photo-patterning [17]. However, these methods require advanced electrospinning setups or bring additional complexity and it is difficult to achieve membranes with pore size in wide range. Recent studies have suggested that various kinds of polymer particles could be formed by electrospraying in the drug delivery applications [18,19]. Xie et al. [20] investigated that polylactide (PLA) microparticles with particle size of around 5 μm can be achieved by electrospraying. Moreover, Valo et al. [21] produced PLA drug-loaded nanoparticles with average diameters of around 200 nm using the technology of electrospraying. By choosing the right parameters, low polydispersity can be obtained with relative standard deviations (RSD) within 2–27% of the average size [20,21]. Ionescu et al. [22] developed a fabrication technique to entrap drug-delivering polystyrene (PS) microspheres within nanofibrous

* Corresponding author at: Key Laboratory of Advanced Textile Materials and Manufacturing Technology of Ministry of Education, Zhejiang Sci-Tech University, Hangzhou 310018, China.
E-mail address: jxiong@zstu.edu.cn (J. Xiong).

http://dx.doi.org/10.1016/j.compositesa.2016.09.015
1359-835X/

scaffolds that the composite would exhibit sustained release of multiple model compounds. We found that polyethylene glycol (PEG) microspheres could be electrosprayed into the electrospun nanofibrous scaffolds by dissolving in chloroform to prepare the electrospraying solution, and PEG is very soluble in water which can be selectively leached in composite membranes. Therefore, we designed a hybrid electrostatic system incorporating PEG microspheres into nanofibers to fabricate 3D nanofibrous scaffolds with larger pore size (Fig. 1). In this system, PEG microspheres fabricated by electrospraying were entrapped into SF/gelatin nanofibrous membranes, consisted of electrospraying and traditional electrospinning that resembles co-electrospinning [23,24]. Selective removal of the sacrificial PEG microspheres was used to improve cell infiltration in the electrospun membrane formed of closely packed nanofibers. Pore size within nanofibrous scaffolds had a huge increase which could reach tens of microns by focusing on changing the blending ratio and optimizing the electrospraying conditions. Furthermore, the mechanical properties of composite scaffolds were evaluated by tensile testing which correlated with the PEG content in composite scaffolds. The electrospun SF/gelatin nanofibers entrapped with PEG microspheres were characterized in terms of pore size, porosity and surface morphology before and after selectively removing the water-soluble PEG inside the scaffolds.

2. Experimental section

2.1. Materials

PEG (molecular weight = 20,000 Da) and gelatin were purchased from Aladdin Inc., Shanghai, China. To obtain the silk fibroin, the silk cocoons were degummed twice through boiling with the aqueous solution of 0.05% (w/w) Na_2CO_3 for 30 min, and then rinsed with distilled water followed by drying at 40 °C overnight to remove the sericin. The degummed silk threads were dissolved in a solvent system of $CaCl_2/H_2O$/EtOH (molar ratio: 1:8:2) at 75 °C for 5 min. The SF solutions was dialyzed in a dialysis tube (Spectra/Por®, MWCO: 8000–14,000, USA) against distilled water at room temperature for 72 h. The dialysis water was changed every 2 h during the day. After dialysis, the SF solution was filtered and then lyophilized to obtain the regenerated SF sponges. All sponges were stored in desiccators before use. Other reagents used in this experimental work are all analytical grade reagents.

2.2. Tailoring of electrosprayed microspheres

Briefly, different amounts of PEG were dissolved in chloroform under continuously magnetic stirring at 30 °C for 2 h. A series of such polymer solutions were prepared by varying the concentration of PEG from 10, 20, 30 to 40 wt%. The homogeneous solution was transferred into a 10 mL syringe fitted with a stainless steel 18G needle that served as a charged spinneret. A high voltage (15 kV) was applied at the tip of the needle by a high voltage power supply (DW-P303-1ACF0 China) and the grounded collector (aluminum foil) located at a distance of 14 cm from the spinneret. PEG microparticles were electrosprayed by varying pump (KDS100, KD Scientific Inc., USA) feed flow rate from the solution of chloroform. A variety of size and shapes of PEG particles were investigated by changing the concentration and feed flow rate of electrospraying. The optimum electrospraying conditions were chosen in order to obtain steady formation of spherical PEG microparticles. Identical amount of electrospraying PEG particles were deposited on the aluminum foil through controlling deposition time of different electrospraying conditions in order to obtain samples of comparable uniformity for the following analysis.

The morphology of the electrospraying particles was characterized using a scanning electron microscope (SEM). Gold (5 nm) was sputtered on all samples using a scanning electron microscope coating unit (E5100) from Polaron Equipment Limited. For each sample, the average diameter and standard deviation of microparticles were calculated from the diameter measured from 50 microparticles in 5 randomly selected areas. The analysis of obtained SEM images was accomplished by Image processing software (Image-pro, NIH).

2.3. Simultaneously electrospinning and electrospraying

SF/gelatin nanofibers were electrospun from a solution of formic acid (FA)/Hexafluoroisopropanol (HFIP). The optimal ratio of SF and gelatin was investigated in previous experimental work

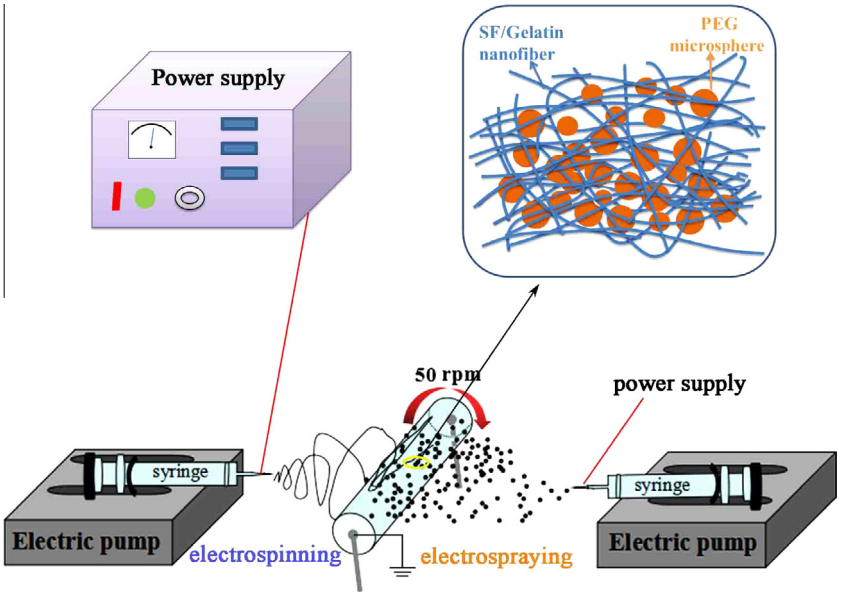

Fig. 1. Schematic illustration of the experimental simultaneously electrospinning and electrospraying setup. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)