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Influence of self-assembly regenerated silk fibroin nanofibers on the properties of electrospun materials

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Abstract. In this study, self-assembly regenerated silk fibroin (RSF) nanofibers were prepared and observed by Atomic Force Microscope (AFM). Then RSF films containing nanospheres and nanofibers were prepared and dissolved with poly (L-lactide-co-ε-caprolactone) (PLCL) with a blending ratio of 30/70 in hexafluoro-2-propanol (HFIP). In order to determine whether different nanostructures in the solution influence the morphological, structural, and mechanical properties of the final electrospun materials, flat membranes were prepared and characterized by Scanning Electron Microscope (SEM), Fourier Transform Infrared (FT-IR), and mechanical testing. The secondary structure of as-spun materials with RSF nanofibers were not changed, however, the diameter of electrospun fibers decreased and tensile strength and elongation at breaks increased. Electrospun materials with RSF nanofibers have the potential to be used for skin, cartilage, and blood vessels because of their biocompatibility and improved mechanical properties.

Keywords: Regenerated silk fibroin, electrospinning, self-assembly, nanostructure, artificial blood vessels

1. Introduction

Silk is one of the strongest natural fibers, featuring an unusual combination of impressive strength and breaking strain [1]. It is indicated that the superior mechanical properties of silk are due to its sophisticated hierarchical structure, which is formed by self-assembly of RSF molecules into nanoscale components [2]. Studies have also revealed that the nanoscale components of silk structures play a fundamental role in achieving great strength, stiffness and resilience on a macro scale [2].

The applications of silk-based materials have expanded from conventional textiles to high-technology fields, including optical devices, tissue engineering, drug release systems, etc. [3, 4]. For applications in the tissue engineering field, silk-based scaffolds for skin, cartilage, artificial blood vessels, etc., can be prepared through casting and electrospinning, as well as with freeze-drying methods. A challenge remains to further improve silk biocompatibility and mechanical adaptability [4]. Inspired by the natural hierarchical structure of silk, the addition of silk nanoscale components into

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silk-based scaffolds may improve its mechanical properties.

Generally, regenerated silk fibroin (RSF) is used to prepare silk-based scaffolds. RSF solution can be obtained by degumming sericin, dissolving the remaining fibroin in LiBr solution, dialyzing the solution against distilled water, and then centrifugation to remove the silk aggregates formed during the process. The fresh RSF solution primarily consists of nanospheres, however, nanofibers are formed in the solution through self-assembling after heat treatment and dilution [5]. In order to determine the effects of self-assembly nanofibers on scaffold properties, fresh RSF solution and solutions with self-assembly nanofibers were each blended with poly (L-lactide-co-ε-caprolactone) (PLCL) for electrospinning as flat membranes. Morphological, structural, and mechanical properties of the final electrospun materials were characterized. PLCL, a copolymer ring opening polymerized by the monomers of PLLA and PCL, exhibits a rubber-like elasticity and satisfying biocompatibility. Scaffolds composed of RSF nanofibers and PLCL are a promising material for blood vessel tissue engineering. Therefore PLCL was selected as the blending agent in this study.

2. Materials and methods

2.1. Preparation of RSF solution

Bombyx mori regenerated fibroin solution was prepared according to previously published procedures [6]. Cocoons were boiled for 20 minutes in an aqueous solution of 0.02 mol/L Na_2CO_3 and then rinsed thoroughly with distilled water to extract the sericin proteins. After drying, the extracted RSF was dissolved in 9.3 mol/L LiBr solution (Sigma-Aldrich, St. Louis, MO) at 60℃ for four hours, yielding a 20% (w/v) solution. This solution was then dialyzed against distilled water, using Slide-a-Lyzer dialysis cassettes (Pierce, MWCO 3500) for 72 hours to remove salt. This solution was centrifuged at 9000 rpm for 20 minutes at 4℃ to remove silk aggregates formed during the process. The final concentration of aqueous silk solution was ~7 wt%, determined by weighing the remaining solid after drying.

2.2. Preparation of RSF nanofiber solutions

A certain amount of original RSF solution was placed in an oven at 60℃ and concentrated to approximately 20%. It was then diluted to 2%, sealed and incubated at 60℃ for 3-4 days, obtaining a gel-like material with abundant nanofibers. The RSF nanofiber solutions were then obtained by ultrasonic dispersion.

2.3. Preparation of polymer solutions with and without RSF nanofibers

For preparing polymer solutions without self-assembly nanofibers, solid RSF film was obtained by drying a 3 wt% RSF solution (obtained by diluting the original RSF solution with deionized water) at room temperature. Solid RSF film and PLCL ($M_w = 154000$ g/mol, Dai-gang Biomaterial Co. Ltd., China) were combined at a weight proportion of 30/70, and were then dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, molecular weight=68.04) (Fluorochem Ltd, Unit 14, UK) at room temperature for more than 12 hours. The concentration of the final polymer solutions was 6 wt%.

For preparation of polymer solutions with self-assembly nanofibers, the original RSF solution and RSF nanofiber solution were blended to obtain solutions with a concentration of 3 wt%, which were

then cast on glass culture dishes to form solid RSF films. Next, the solid RSF film containing self-assembly nanofibers and PLCL were combined at a weight proportion of 30/70, and were then dissolved thoroughly in HFIP at room temperature. The concentration of the final polymer solutions was also 6 wt%.

2.4. Electrospinning

Electrospinning was performed according to previously reported procedures [6]. Polymer solution was placed in a syringe with a 0.7 mm outer diameter pinhead. A positive voltage of 15 kV was applied to the pinhead and shifted at a speed of 20 mm/s. The delivery speed of the solution was 0.01 m/s. The electrospun fibers were collected as flat membranes on aluminum-foil. The collecting distance of the flat membranes was 16 cm.

2.5. Characterization

2.5.1. Atomic force microscopy (AFM)

In order to prepare AFM samples, a RSF solution of 0.1 wt% was prepared by diluting the original RSF solution and RSF nanofiber solution with deionized water. Next, 2 μL of the diluted RSF solution was dropped onto freshly cleaved 4 × 4 mm^2 mica surfaces. The morphology of RSF in water was observed by AFM (Veeco, Nanoscope V) in air. A 225 μm silicon cantilever with a spring constant of 3 N/m was used in tapping mode at a 0.5-1 Hz scan rate.

2.5.2. Scanning electron microscopy (SEM)

The morphology of flat membranes was observed under SEM (Hitachi S-4800, Japan) using an accelerating voltage of 3 kV. Specimens were mounted on small copper plates before being coated with gold.

2.5.3. Fiber diameter calculation

First, SEM photoes were taken of three parts of each sample. Then, the diameter of 50 fibers on the three SEM photoes was measured by ImageJ software. Finally, the average fiber diameter of each sample was calculated.

2.5.4. Fourier transform infrared (FTIR)

FTIR spectra were obtained using a Magna spectrometer (NicoLET5700, America) in the spectral region of 400-4000 cm^{-1}. The powdered RSF electrospun materials were pressed into potassium bromide (KBr) pellets prior to data collection.

2.5.5. Mechanical testing

In order to determine the mechanical properties of the flat electrospun membranes, an Instron electronic strength tester (Instron 3365) was used at an atmosphere of 23±3℃, and 70±5% RH. Strips of 5 × 1 cm were fixed on a paper frame and then mounted on the tester; tensile properties were obtained by measuring four specimens. The drawing speed of the tester was 10 mm/s. Young's modulus, tensile strength and elongation at breaks were calculated based on the obtained data.

3. Results and discussions

3.1. Nanostructures of the RSF solutions

As shown in Figure 1A, the original RSF solutions primarily consist of nanoshperes which were formed by chain folding and hydrophobic interactions [7]. When the original RSF solution was concentrated, diluted and then incubated, providing sufficient time and adapting circumstance for self-assembly, a gel-like material was formed macroscopically. In order to observe the nanostructure under AFM, the gel-like material was dispersed into solution by ultrasonification, and then diluted to 0.1%. As shown in Figure 1B, nanofibers were formed during the self-assembly process. The nanofiber structure remained stable even after ultrasonification.

3.2. Morphology of flat electrospun membranes

Both of the polymer solutions, with and without self-assembly nanofibers, can be electrospun successively. The morphology of flat electrospun membranes are shown in Figures 2A and 2B. From the solution of PLCL blended with the original RSF solution, the flat electrospun membranes were composed of smooth fibers with an average diameter of 963±276 nm. From the solution of PLCL blended with RSF nanofiber solution, the average diameter of the electrospun nanofibers was 630±273 nm, which was significantly thinner, indicating that it was much easier for a solution containing nanofibers to form thin fibers.

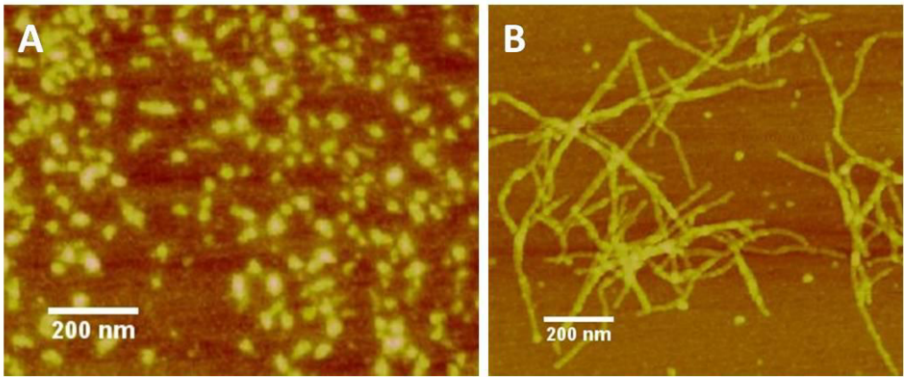

Fig. 1. Nanostructure of (A) original RSF solution and (B) RSF nanofiber solution.

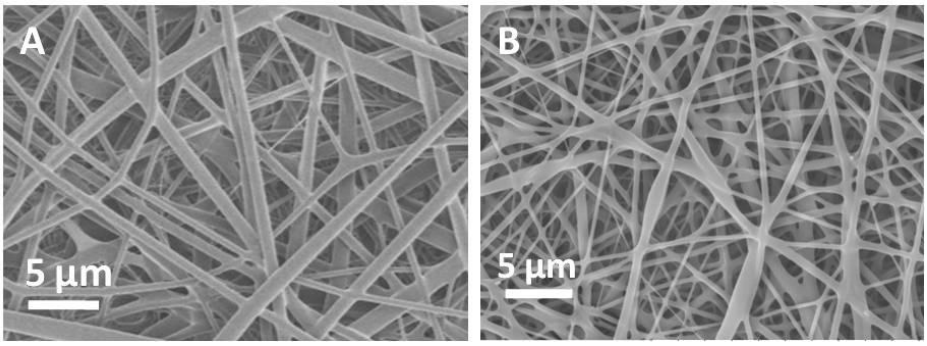

Fig. 2. Flat membranes (A) without RSF self-assembly nanofibers and (B) with RSF self-assembly nanofibers.

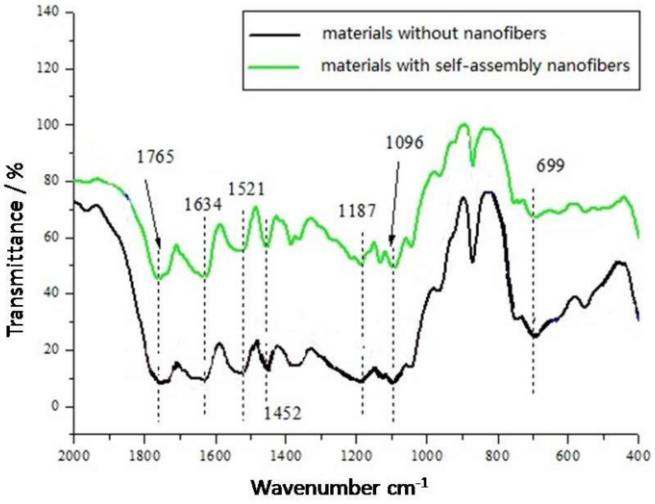

Fig. 3. FTIR spectra of flat electrospun membranes derived from original RSF and RSF nanofiber solutions.

Table 1

Mechanical properties of flat electrospun membranes with and without self-assembly nanofibers

	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
Materials without nanofibers	0.44	0.20±0.07	14.9±3.0
Materials with self-assembly nanofibers	0.45	0.23±0.04	47.3±5.5

3.3. Secondary structure

FTIR analysis was utilized to study structural changes in the flat electrospun membranes prepared from solutions with and without self-assembly nanofibers after 75% ethanol post-treatment (Figure 3). The electrospun flat membranes prepared from the two different blending solutions showed similar characteristic peaks of silk II, at 1634 cm^{-1} in amide I and 1521 cm^{-1} in amide II. This indicated that the secondary structure of the flat membranes was not affected by the addition of self-assembly nanofibers.

3.4. Mechanical properties

The thickness of the flat electrospun membranes were measured three times each with a thickness gauge; the average thickness was 0.45 mm and 0.44 mm for the flat membranes with and without self-assembly nanofibers, respectively. The mechanical properties of the flat electrospun membranes (Table 1) derived from RSF nanofiber solution displayed a tensile strength of 0.23 Mpa and an elongation at breaks of 47.3%; both values were higher than those of the membranes derived from original RSF solutions. Self-assembly nanofibers acted as a disperse phase, which strengthened the blended material. Meanwhile, there is also a conjugation effect between nanofiber and its surrounding matter, which increases flexibility of the blended material. Therefore, the addition of self-assembly nanofibers can both strengthen and flexibilize the blended materials, and the flexibilization effect is particularly significant. The addition of self-assembly nanofibers is a possible method to improve the strength properties of electrospun materials, although further study is necessary to illustrate the

mechanism.

4. Conclusions

Regenerated silk fibroin (RSF) self-assembly nanofibers were prepared in this study. The nanofibers can be used to improve the mechanical properties and to control the fiber diameter of electrospun materials. Based on our study, the design and preparation of silk-based electrospun scaffolds of adjustable sizes and mechanical properties becomes possible, which could facilitate further application in the fields of regenerative medicine and tissue engineering.

Acknowledgments

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