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Controlling silk fibroin microspheres via molecular weight distribution

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ABSTRACT

Silk fibroin (SF) microspheres were produced by salting out SF solution via the addition of potassium phosphate buffer solution (K_2HPO_4–KH_2PO_4). The morphology, size and polydispersity of SF microspheres were adjusted by changing the molecular weight (MW) distribution and concentration of SF, as well as the ionic strength and pH of the buffer solution. Changing the conditions under which the SF fiber dissolved in the Lithium Boride (LiBr) solution resulted in altering the MW distribution of SF solution. Under optimal salting-out conditions (ionic strength > 0.7 M and pH > 7) and using a smaller and narrower SF MW distribution, SF microspheres with smoother shapes and more uniform sizes were produced. Meanwhile, the size and polydispersity of the microspheres increased when the SF concentration was increased from 0.25 mg/mL to 20 mg/mL. The improved SF microspheres, obtained by altering the distribution of molecular weight, have potential in drug and gene delivery applications.

1. Introduction

Silk from the *Bombyx mori* silkworm is a composite fiber, consisting of two strands of silk fibroin (SF) surrounded by a cementing layer of sericin [1,2]. SF is the major component of this silk fiber, occupying 75% of its total weight [3]. It has been employed in textile industries for several thousand years. Due to its superior mechanical properties, excellent biocompatibility and biodegradability, it has found the application of SF in the fields of biomedical materials, cosmetics, food and pharmaceuticals [4–6].

Regenerated SF obtained after cocoons have been subject to a process of degumming, salt dissolution, dialysis and purification. Regenerated SF is usually used in advanced applications. The regenerated SF can be processed into various forms including gels [7], fibers [8,9], films [10,11], porous scaffolds [12–14], microneedles [15], microspheres [16–21] and nanoparticles [22]; specific forms may be chosen depending on the requirements of certain applications. Recently, micro/nano-sized particles prepared by silk protein have received extensive attention due to promising applications toward antibacterial materials [23], drug delivery [24,25], and gene engineering [26].

Generally, two methods are used to prepare SF microspheres: microemulsion and phase separation. In microemulsion, the emulsion droplets of the aqueous SF solution are formed in the oil phase to control the shape and size of SF particles. However, this process is complicated, as it requires emulsion stabilizers and cross-linkers [19,27,28]. The SF spheres synthesized with this approach have a round and unattached appearance with a relatively large size. Baimark's group has conducted many studies in this topic, producing SF microparticles that were completely spherical under Span80. These SF microparticles were irregular without the attendance of the oil (Span80), but became completely spherical in shape when Span80 was employed in the system; the surface of the SF particles was smooth and their sizes were around 20 μm [28]. The group also produced SF microspheres that had a spherical shape and smooth surface when paraffin was introduced; most of these SF microspheres were 80–150 μm in size [27]. When dichloromethane was applied as the oil phase, the size of these SF microspheres ranged from 45 to 92 μm [19]. However, the obtained SF particles required cleaning to remove organic constituents before further biomedical applications. In contrast, phase separation of SF solution by adding salts or low dielectric organic solvents, as reported by Lammel et al. [29], is a relatively simple method. The salted out microspheres, triggered by the salts, precipitate and aggregate during the phase separation process [22,29,30]. The SF particles were small in size (35–125 nm [22], 486 nm–2.0 μm [29], 0.2–1.5 μm [30]), but the particles preferred to be in adhesion. The size, shape and distribution of the particles (synthesized by polymers or macromolecules) can be determined by their MWs, which can affect the performance of the particles' stiffness [31], degradation [32], and other characteristics. The ability to control SF MW makes it advantageous for use in developing micro- and nanoparticles, with a possibility of directly furthering the possibilities of future applications.

This report investigates changing SF MW distributions by applying different dissolving conditions. The effect of MW on the size and shape of SF microparticles was studied under various salting-out conditions (salt solutions with different ionic strength and pH values). Furthermore, the effect of MW on SF microsphere formation dynamics and mechanisms were

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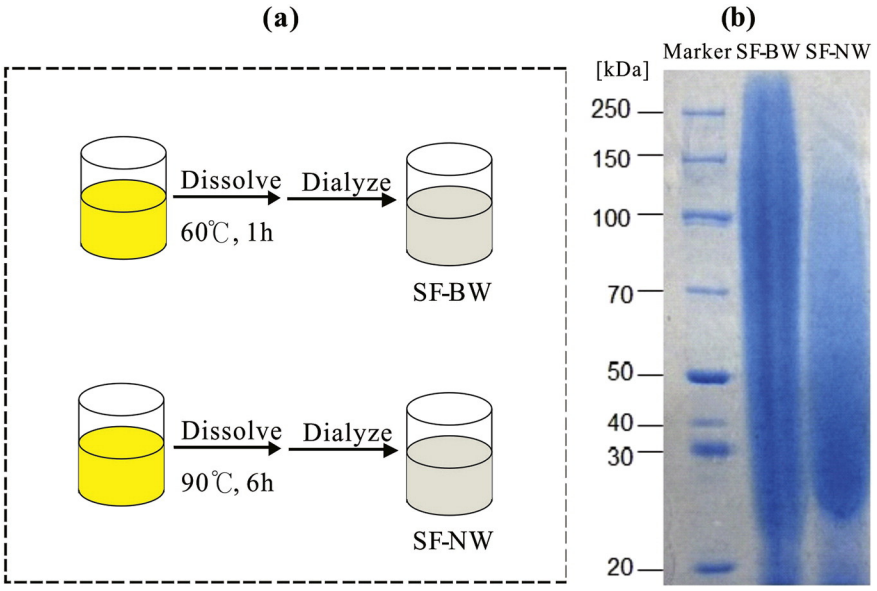

Fig. 1. (a) Schematic procedures of two kinds of aqueous SF dissolved at 60 °C for 1 h (SF–BW denotes broad MW distribution) and 90 °C for 6 h (SF–NW denotes narrow MW distribution), respectively. (b) SDS-PAGE (8%) electrophoresis of SF–BW and SF–NW.

also discussed. The individual SF microparticles studied in this work exhibited relatively smaller sizes and spherical well appearance.

2. Experimental

2.1. Materials

B. mori silkworm cocoons were provided by Jiangsu Huajia Silk Co., Ltd., China. Dialysis membranes were purchased from Shanghai Genestar Biotech Co., Ltd., China. Sodium dodecyl sulfate (SDS), N,N,N′,N′-tetramethylethylenediamine (TEMED), acrylamide β-mercaptoethanol and Coomassie brilliant blue G-250 were obtained from Sigma-Aldrich, USA. Ammonium persulfate (AP), glycine, acetic acid, Na_2CO_3, LiBr, and potassium phosphate were all analytical grade reagents, purchased from Sinopharm Chemical Reagent Suzhou Co. Ltd., China. Unstained broad range protein ladder (5–250 kDa) was purchased from Fermentas, Canada. Reagents in the experiments were used as purchased without further purification.

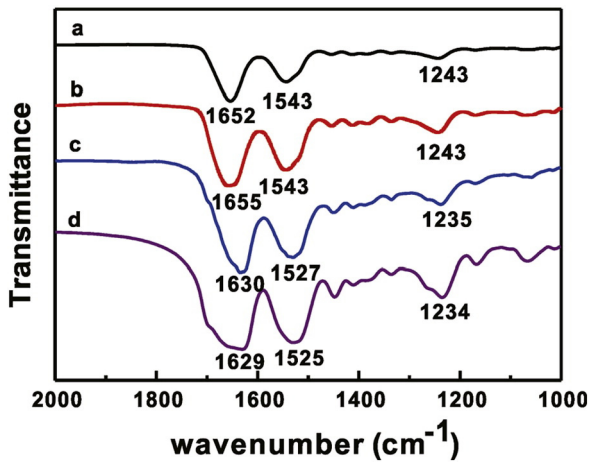

Fig. 2. FTIR spectra of the regenerated SF membrane and SF particles produced by salting out SF solution (concentration of 5 mg/mL) with potassium phosphate (1.25 M, pH = 8): (a) SF–BW membrane; (b) SF–NW membrane; SF particles from SF–BW (c) and SF–NW (d).

2.2. Preparation of purified SF solution

The silk was first degummed to remove the sericin, as described in Refs. [6–18,27–30]. More specifically, the cocoons of *B. mori* silkworm were cut into small pieces, degummed by boiling in a solution of 0.5% (w/w) Na_2CO_3 for 1 h, and rinsed with deionized water 4–5 times to remove sericin residuals. The degummed SF fibers were subsequently dissolved in 10 M LiBr solution at 60 °C for 1 h, and then at 90 °C for 6 h. The SF solution was filtrated by gauze to remove impurities, and dialyzed against deionized water using a cellulose dialysis membrane (MWCO 14000) for 3 days. The solution was centrifuged at 10,000 rpm for 20 min to remove the precipitate. The final concentration of SF solutions can be determined by gravimetric method, which provided a value of approximately 5% (w/w). The SF solutions were stored at 4 °C and diluted with ultrapure water for further usage.

2.3. Fabrication of SF microspheres

SF microspheres were prepared by salting out SF solution with the K_2HPO_4–KH_2PO_4 solution [29]. The ionic strength and pH value of potassium phosphate buffer solution could be changed via mixing K_2HPO_4 and KH_2PO_4 in different volume ratios. The SF solution was mixed rapidly with potassium phosphate in volumetric ratios of 1:5 using a pipette; the mixed solution was stirred at 500 rpm for 5 min at room temperature. The mixed solution was stored at the 4 °C for 2 h, and then placed at room temperature for 12 h. The dispersion of microspheres was centrifuged at 6000 rpm for 15 min, with deionized water replacing the supernatant, and redispersed by sonication. The above process was repeated three times. The unevenness of the mixing process generated some large aggregates and precipitations that settle down together with the microspheres by the above centrifugation. To remove the larger particles, the microsphere solution was centrifuged again at 500 rpm for 5 min. The final microsphere solution was stored at 4 °C before use.

2.4. Characterization

2.4.1. SDS-PAGE electrophoresis

The MW distribution of SF was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), as described by Laemmli [33].