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Regeneration of high-quality silk fibroin fiber by wet spinning from $CaCl_2$–formic acid solvent

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ABSTRACT

Silks spun by silkworms and spiders feature outstanding mechanical properties despite being spun under benign conditions. The superior physical properties of silk are closely related to its complicated hierarchical structures constructed from nanoscale building blocks, such as nanocrystals and nanofibrils. Here, we report a novel silk dissolution behavior, which preserved nanofibrils in $CaCl_2$–formic acid solution, that enables spinning of high-quality fibers with a hierarchical structure. This process is characterized by simplicity, high efficiency, low cost, environmental compatibility and large-scale industrialization potential, as well as having utility and potential for the recycling of silk waste and the production of silk-based functional materials.

1. Introduction

Even though silk is composed of protein and is spun in environmentally benign conditions, natural selection and evolution have endowed it with exceptional mechanical properties that outperform most known industrial fibers [1,2]. *Bombyx mori* silk has long been used to fabricate luxurious fabrics due to its amazing fineness and luster [2]. More recently, silk has shown a bright future for applications in various high-tech areas due to the possibilities offered by the states and properties of regenerated silk materials [3,4].

The dissolution of silk is a critical step in producing regenerated silk fibroin (RSF) fibers with superior performance [5–7]. Native silk has been dissolved in H_3PO_4 [8], H_3PO_4–formic acid (FA) [9], LiBr (self-dialysis) [10] or ionic liquids [11] for direct spinning of fibers; although these processes are simple the resulting fibers perform poorly. In addition, regenerated silk materials (films or lyophilized sponges) have also been dissolved in 1,1,1,3,3,3, -hexafluoro-2-propanol [12,13], FA [14], trifluoroacetic acid [15], *N*-methyl morpholine *N*-oxide [16] and water after concentration [17,18] to achieve spin dopes used for fiber formation [19,20]. However, these spinning dopes require complicated preparation processes, and most of these solvents either degrade the silk fibroin or are expensive, and an organic solvent or ammonium sulfate coagulation bath is necessary, which restricts the production of RSF fibers on an industrial scale [6,11,17]. Moreover, despite extensive study, the regeneration of fibers with properties that match the mechanical features of native silk fibers remains a significant challenge, probably due to the absence of the complex hierarchical structure [21] that has recently been recognized as the key to the high performance of native silk [22–24].

In order to recycle and utilize silk waste, as well as achieve large-scale production of functional materials, it is critical to be able to regenerate high-performance silk fibers through a simple but efficient spinning process. In this work, we report a novel wet-spinning process for producing fibers by dissolving silk in $CaCl_2$–FA solution instead of $CaCl_2$–ethanol–H_2O which has been widely used to dissolve silk [25–27]. The resulting fibers exhibited hierarchical fibrillar structures and also showed higher strength and extensibility than native silk. Compared to traditional methods, this spinning process has the following characteristics: (i) silk was dissolved in $CaCl_2$–FA directly for spinning without any further treatment; (ii) silk collapsed into nanofibrils instead of isolated molecules in the $CaCl_2$–FA solution; (iii) the process employs water as the coagulation bath at room temperature.

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2. Experimental methods

2.1. Preparation of spin solution

The spin dope was prepared from *B. mori* silk dissolved in $CaCl_2$–FA. The cocoons were boiled for 60 min in an aqueous solution of Na_2CO_3 (0.05% w/v) and then rinsed thoroughly with distilled water to extract the sericin proteins. The dried degummed silk was then directly dissolved in $CaCl_2$–FA with a $CaCl_2$ concentration of 4% (w/v) for 3 h at room temperature to prepare a 15% (w/v) SF solution. This SF–$CaCl_2$–FA solution was used for wet-spinning.

For comparison, a traditional silk dope was prepared as follows: the degummed silk was dissolved in 9.3 M LiBr solution for 4 h to attain fibroin solution; the fibroin–LiBr solution (20% w/v) was dialyzed against deionized water for 3 days to remove LiBr; dialyzed fibroin solution was lyophilized for 2 days to generate dried silk materials; finally, the dried silk materials were dissolved in FA for 3 h to prepare 15% (w/v) SF solution for wet-spinning. This process for preparing traditional silk dope is time consuming and complex due to the multiple steps. The preparation of spin dope requires 3 h with SF–$CaCl_2$–FA and 127 h with SF–FA. Moreover, the weight loss of silk is about 0% and 20% for the SF–$CaCl_2$–FA and SF–FA solution preparations, respectively.

2.2. Wet-spinning

A schematic of the wet-spinning apparatus built and used for spinning RSF fibers in this study is shown in Fig. S1. All experiments were conducted at room temperature. A syringe pump was used to extrude the SF solution at 10 ml h^{-1} through a syringe needle (0.6 mm in internal diameter) into a flowing water coagulant bath (methanol was used for SF–FA solution) at room temperature. For environmental protection, excess FA solution leaving the coagulant bath was neutralized by NaOH in a neutralization bath. The take-up rate of the collector was 20 cm min^{-1}. The resulting SF fibers (termed "as-spun fiber") on the collector were immersed in water overnight for further solidification and removal of FA. The dried silk fibers were immersed in water for 30 min for further drawing between calipers in a wet state. Draw-ratios of 2, 3 and 4 were defined as ratio of the fiber length after drawing to the original length, and are hereinafter referred to as RSF-2×, RSF-3×, RSF-4×.

2.3. SEM imaging

For scanning electron microscopy (SEM) imaging, 2 μl SF–$CaCl_2$–FA solution (1 μg ml^{-1}) was dropped on to a silica plate, and left to dry. Samples were sputter-coated with a gold layer prior to imaging. The morphology of the nanofibrils, degummed silk and wet-spun fibers was observed using a scanning electron microscope (Hitachi S-520, Japan) at 20 °C, 60 relative humidity.

2.4. X-ray diffraction

To investigate the structural change of silk during dissolution in $CaCl_2$–FA and regeneration by wet-spinning, we performed X-ray diffraction (XRD) analysis on native silk, dry-spun silk and wet-spun silk. The theta–theta diffractometer (X'Pert Pro MPD, PANalytical Company, the Netherlands) with Cu K_α radiation working at 40 kV and 40 mA was used to record X-ray diffractograms in the interval $2\theta = 2°–45°$ at a scan rate of 2K min^{-1}.

2.5. Fourier transform infrared spectroscopy

Structural changes in the silk–$CaCl_2$–FA solution and wet-spun fibers were analyzed by Fourier transform infrared spectroscopy (FTIR) using a Magna spectrometer (NicoLET5700, USA) in the spectral region of 400–4000 cm^{-1}. For each measurement 32 scans were recorded at a resolution of 4 cm^{-1}. Prior to data collection, the silk–$CaCl_2$–FA was directly dropped onto potassium bromide (KBr) plates, and the powdered wet-spun silk fibers were pressed into KBr plates.

2.6. Raman analysis

Raman spectra were recorded on single silk fibers with a Dilor LabRam-1B Raman microscope (Dilor, Lille, France) using a He–Ne laser with an excitation wavelength of 632.8 nm. The laser beam was polarized either parallel or vertical to the long axis of the fiber. All the spectra were normalized by the intensity at 1615 cm^{-1} assigned to the phenyl group, which is thought to arise mainly from the tyrosine residues in the silk.

2.7. Mechanical testing

An Instron 5565 mechanical testing instrument (Instron, Norwood, MA) with a 5 N load cell was used for single fiber testing (25 ± 0.5 °C; 60 ± 5% relative humidity; gauge length: 10 mm; cross-head speed: 10 mm min^{-1}). At least 10 measurements for each sample were performed in the testing. The significance of difference of RSF-4× from native silk in terms of strength, strain and Young's modulus was obtained by one-way ANOVA.

3. Results and discussion

3.1. Description of the wet-spinning process

Fig. 1 shows the simple fiber regeneration process directly from SF–$CaCl_2$–FA solution, which is similar to the ionic liquid-spinning process [11]. Native silk is composed of multilevel fibrils with diameters reported to be in the range from 20 to 170 nm [28,29]. Ultrasonic impact can thoroughly disintegrate micron-sized natural silk fibers into nanofibrils due to the relatively weak interfaces among the fibrils [29]. We also found that $CaCl_2$–FA gradually disintegrates macrofibers into microfibers, then into nanofibrils (Fig. 1b) [30]. This process took 3 h or so, and the resulting solution remained stable for at least 24 h at room temperature based on a slight decrease in viscosity (unpublished results), providing sufficient time for the wet spinning. The peak and distribution of dynamic light scattering (DLS) provided evidence for the presence of nanofibril structure (Fig. S2). Although DLS is not suitable for the characterization of the fibrous structure, the broader size distribution of silk in $CaCl_2$–FA implied a different aggregate structure, confirming the nanofibril preservation observed by SEM [31]. It is suggested that the dissolution of silk might be inhibited or even suppressed when its size falls to the nanofibril level. Therefore, these nanofibril structures should be resistant to dissolution caused by $CaCl_2$–FA on account of their sizes and should remain relatively stable in this solvent. The self-assembled silk nanofibrils seemed also to be dispersed instead of being destroyed in FA, indicating the stability of the silk nanostructure in FA [32].

After wet-spinning, these nanofibrils easily reassembled into fibers due to physical shear and coagulation in water. This process differs from the previous works in which silk was dissolved into molecules of smaller size [33]. We also report here for the first time the use of water for the coagulation bath, which has the advantage of being inexpensive, simple in operation and environmentally

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