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Original Research

Role of humidity on the structures and properties of regenerated silk fibers

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Abstract

Silk fiber was processed from highly concentrated spinning dope to solid fibers along with water removal. To understand the mechanism of water removal during silk fiber spinning process, a microfluidic chip was designed and applied to investigate the structures and mechanical properties of two kinds of regenerated silk fibroin fibers dry-spun at different relative humidity. The experimental results showed that the diameters of the fibers spun at 40% RH are always larger than the fibers spun at 50% RH due to different removal rates of water. The fibers spun at low humidity contain more β-sheet structure and lower degree of chain orientation and crystalline orientation. These results indicate that the fast phase transition of silk fibroin from sol–gel to silk fiber undergoes with rapid water removal and higher fiber orientation relates to more residue water and drawing force.

Keywords: Dry spinning; Relative humidity; Biomimetic; Regenerated silk fibroin; Wide angle X-ray diffraction

1. Introduction

Water plays a significant role in both assembly of silk protein and formation of silk fiber. Silk fibroin heavy chain is made up of alternate arrays of hydrophilic and hydrophobic blocks [1]. On one hand, the interactions between water and hydrophilic blocks make silk protein maintain soluble and avoid crystallization before spinning process. On the other hand, the increase of fibroin concentration facilitates the interactions between hydrophobic blocks and induces the formation of silk I structure [2]. Rapid removal of water during the shear and elongational flow results in a liquid–solid transition [3–5]. The phase transition from silk I structure to silk II structure is attributed to the removal of water molecules around the silk chains and the application of shear and stretching [6]. However, further loss of water after silk fiber exiting spigot has not been studied in detail and usually considered unnecessary.

Water also has a significant influence on the crystallization process of silk fibroin film from aqueous solutions. Water removal above the glass transition temperature T_g promotes the phase transition from noncrystalline random coils or α-helices to β-sheet crystals [7,8]. Moreover, the phase transition from liquid-crystalline to solid fiber associates with the loss of partial water in the spinning dope [9].

Shear and elongational flow is another critical factor in the fiber formation. The spinning ducts of spider and silkworm are similarly tapered geometry [10]. Although their spinning dopes were subjected to distinct shear and extensional flow, the role of flow has been proved to be essential to the silk protein assembly and fiber formation. Recombinant spider silk protein can only be processed into fiber in the presence of elongational flow [11]. The Rapid extensional flow in the distal part of spinning duct of silkworm promotes the aggregate of silk fibroin molecules and formation of silk II structure, following by the increase of birefringence of silk fibroin [6]. And the shear induces the assembly of silk fibroin and initiates the formation of fibril [12]. Moreover, the shear forces are thought to be involved in the formation of liquid crystalline phase [13].

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Recent advances in understanding of native spinning process have shown that the extraordinary properties of silk fiber are controlled by a combination of changes in chemistry and extrusion conditions [2,14,15]. And the wet spinning makes use of different solvents and post-spinning drawing to improve the microstructure and mechanical properties of regenerated silk fibers [16–18].

Microfluidic is a newly advocating technique to fabricate microfibers [19–21]. Our previous work demonstrated the probability of biomimic spinning using a microfluidic chip from regenerated silk fibroin (RSF) aqueous solutions [22]. In this study, we fabricated a new microfluidic chip with different geometry, which is designed by mimicking the geometry of silkworm silk gland and spinning duct. Especially, the role of water on the formation of RSF fibers was investigated by studying the effects of RSF concentration and relative humidity (RH) on the structures and properties of RSF fibers.

2. Experimental

2.1. Spinning apparatus and fiber formation

A dry-spinning apparatus was constructed with a progressively narrowing fluidic channel. Due to the high concentration of RSF spinning dope, the width of the shear segment of the microchannel shown in Fig. 1 was enlarged to 270 μm, which is 5 times as wider as the natural spinning duct [6]. The RSF solution was introduced through the microfluidic channel at a flow rate of 2 μL $\min^{-1}$. The spinning rate was controlled at 3 cm s^{-1}. The spinning conditions were kept at (23 ± 1) °C and constant relative humidity (RH) of 40 ± 5%, 50 ± 5% or 60 ± 5%, respectively. After dehydration in the dry cabinet, as-spun fibers were stretched at the draw ratios of 4 and 0.9 mm s^{-1} in 80 (v/v)% ethanol aqueous solution, which changes the fibroin conformation from random coil/α-helix to β-sheet. In addition, the post-drawing process induces the orientation of the β-sheet crystallites, which contributed to the improved breaking stress of the fiber [23]. The post-drawn fibers were then immersed in the aqueous solution for 2 h at a fixed length [24]. For sample designation, we use C and R to present concentration of RSF in the spinning dope and relative humidity, respectively. For example, the fibers spun from 44 wt% RSF spinning dope at 40 ± 5% RH was designated as C44R40, which was post-drawn at a draw ratio of 4. Other fibers were renamed as C44R50, C47R40, and C47R50 according to the same description.

In Fig. 1B, the microfluidic channel is composed of three segments, including dope store segment (yellow), elongation segment (without color) and shear segment (blue). The blue dot at the left of the channel indicates inlet. The elongation curves were fitted using the second order exponential decay function ($Y=A(1/(1+\exp(BX))+C(1/(1+\exp(DX))$, $A=238$, $B=6.18\mathrm{E}-05$, $C=588$, $D=0.003$) [6]. This curve is similar to the silk gland of silkworm circled in Fig. 1A.

2.2. Spinning dope

Bombyx mori cocoons were firstly degummed twice using Na_2CO_3 solution. Then the dried degummed fibers were dissolved in a 9.0 M LiBr aqueous solution at 40 °C for 2 h. The solution was dialyzed afterwards in deionized water for 3 days. When the RSF aqueous solution was concentrated to 20 (w/w) %, $CaCl_2$ aqueous solution of 3 M was added into the solution to 1.0 mmol/g calcium ion concentration in RSF solution, which was then concentrated by forced air flow to 38–47 wt%. The purpose of adding $CaCl_2$ is to promote the RSF conformation transition from random coil/α-helix to β-sheet and make the RSF solution stable [25,26].

2.3. Mechanical testing

Mechanical properties of the fibers were tested using an Instron 5565 material testing instrument (Instron Ltd., High Wyconbe, UK) with a load cell of 2.5 N, at (24 ± 1)°C and (50 ± 5) % RH. The extension rate was set to 2 mm $\min^{-1}$ with a gauge length of 10 mm. At least 15 measurements for each sample were performed in the testing. The diameter and birefringence of the fibers were tested using a BX-51 polarizing microscope (Olympus, Japan) equipped with a U-CTB Berek compensator. The diameter of the fiber was obtained from more than 10 points distributed along the fiber axis in optical microphotographs.

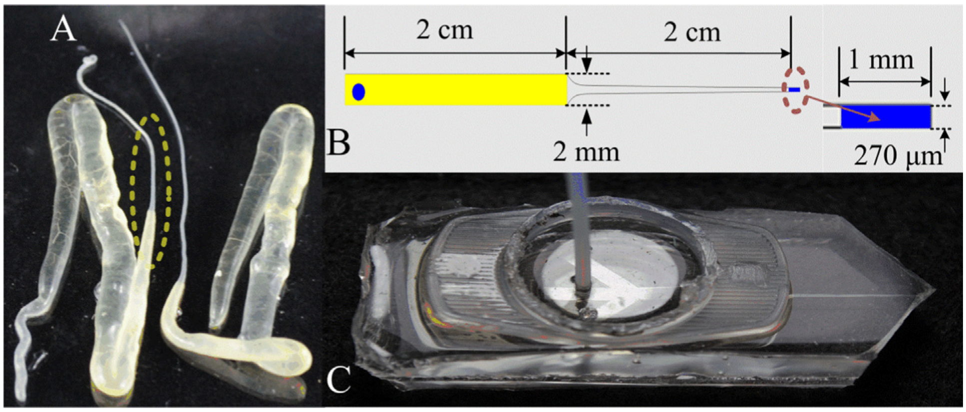

Fig. 1. (A) Silk glands of silkworm and (B) a biomimetic microchannel in (C) a microfluidic chip used for fiber spinning.