

# Regenerated silk fiber wet spinning from an ionic liquid solution

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Regenerated silk fibroin from *Bombyx mori* silkworms was extruded into fibers from a 1-ethyl-3-methylimidazolium chloride ionic liquid solvent system; the drawn fibers rinsed in methanol exhibit alignment of the  $\beta$ -sheet crystallites along the fiber axis.

The impressive properties of naturally spun silk from the *Bombyx mori* silkworm have led to wide use of silk throughout history. In an effort to utilize waste material and produce specialty materials, alternative silk processing techniques have been investigated for silk.<sup>1</sup> In this work, we examine the feasibility of spinning silk fibers from an ionic liquid solution containing silk fibroin.

Artificial spinning methods for silk fibroin have typically fallen into the categories of wet spinning<sup>2–8</sup> and electrospinning.<sup>9–12</sup> Wet spinning has the advantage of producing reelable fiber that can be drawn and tested for mechanical properties. Electrospinning can produce extremely fine fibers in the form of a non-woven mat, however, mechanical testing of individual fibers is not feasible. These electrospun mats can be used as scaffolds for biomedical applications.<sup>1,9</sup>

In previously reported results, spin dopes have been formed with silk fibroin dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP),<sup>2–6,10</sup> hexafluoroacetone (HFA),<sup>4,11</sup> formic acid,<sup>7,8,12</sup> trifluoroacetic acid (TFA),<sup>7</sup> and water mixed with polyethylene oxide (PEO).<sup>9</sup> All of these spin dopes require a time-consuming, multistep process, where the silk fibroin is first washed of the glue-like sericin covering the fiber, dissolved in a salt based solvent, dialysed, and dried to eliminate the  $\beta$ -sheet crystal structure before the fibroin is soluble in the spinning solvent. Jin *et al.* used a process that stopped after the dialysis step, where they introduce PEO to allow for electrospinning.<sup>9</sup> It has been shown previously that silk fibroin is directly soluble in organic-based ionic liquids, both with or without the sericin present.<sup>13</sup> These ionic liquid silk solutions were used to cast films of silk. As a spin dope, ionic liquids exhibit several advantages over the aforementioned spin dopes, such as negligible volatility and ease of solvent recovery.

The approach to wet spinning of silk fibroin has varied in terms of equipment. Trabbic and Yager,<sup>3</sup> Ha *et al.*,<sup>7</sup> and Um *et al.*<sup>8</sup> used syringe pumps with hypodermic needles to spin their dopes.

Yao *et al.*<sup>4</sup> and Zhao *et al.*<sup>5</sup> used a cylinder pressurized with N<sub>2</sub> gas to force their spin dopes through a spinneret. Liivak *et al.*<sup>6</sup> also used a syringe pump, however they used a tapered spinneret etched in silicon to better control the fiber crystallite orientation. All of these spinning techniques utilized a methanol coagulation bath to remove the spinning solvent and induce crystallization in the fibers. The subsequent post-spin fiber drawing was conducted after soaking the fibers overnight in a methanol bath while the fibers were wet with methanol<sup>3,6,7</sup> or while the fibers were in a water bath.<sup>4,5,8</sup> Some of the fibers were also steam annealed.<sup>4,5</sup> The drawing and annealing improved both the maximum modulus and the strain to break of the fibers.

For this work, the spin dope was prepared from silkworm cocoon silk dissolved in the ionic liquid 1-ethyl-3-methylimidazolium chloride (EMIC). The silk for this work was grown in house with silkworms raised on a diet consisting exclusively of Silkworm Chow (Mulberry Farms, Fallbrook, CA). The cocoons were harvested between two and seven days of spinning by cutting the cocoons to release the pupae unharmed, which reduces the contaminants in the silk. To remove the sericin, the cocoons were boiled in a 50 mM Na<sub>2</sub>CO<sub>3</sub> and 50 mM Na<sub>2</sub>EDTA solution, thoroughly rinsed in distilled, deionized water, and lyophilized. The dried silk was mixed with the dry EMIC to make a 10% (w/w) spin dope solution. To dissolve the silk, the spin dope was heated at 95 °C and stirred while exposed to ambient air with a 20% RH. The water content of the final spin dope cooled to room temperature was 1.62 ± 2.00% (w/w) as determined *via* headspace analysis using a Mettler Toledo DL39 Karl Fischer coulometer connected to a Stromboli<sup>®</sup> oven sample changer. Spin dope was also prepared under a dry N<sub>2</sub> atmosphere, however, the dry spin dope was glassy at room temperature and unusable in our spinning apparatus.

For the spinning equipment, we used a glass syringe fitted with a 26 gauge, blunt tip needle with an inside diameter of 0.26 mm and an overall length of 23 mm. The spin dope was placed into the syringe while hot, and the air was removed. The spinning was performed by extruding the spin dope at a rate of approximately 3 m min<sup>-1</sup> across a 50 mm air gap into a coagulation bath. The extruded fiber swelled to a diameter of about 1 mm before entering the coagulation bath.

Several solvents were tested as coagulation baths. Methanol, acetonitrile, and water were previously tested as coagulation solvents for silk fibroin dissolved in the ionic liquid 1-butyl-3-methylimidazolium chloride (BMIC).<sup>13</sup> The films cast from the ionic liquid solution had varied properties: the methanol resulted in a clear, crystalline film, the acetonitrile resulted in light-scattering, amorphous film, and the water dissolved the film. The coagulation bath solvent effects for the spun fibers are listed in

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**Table 1** Coagulation bath solvent effect

Solvent	Effect on spin dope
Methanol	Solidified, clear fibers
Acetonitrile	Solidified, white crusted, brittle fibers
Water	Dissolved with residual, gossamer fibers <sup>a</sup>
Ethyl acetate	Droplet formation <sup>b</sup>
Acetone	Droplet formation <sup>b</sup>
Hexanes	Droplet formation <sup>b</sup>

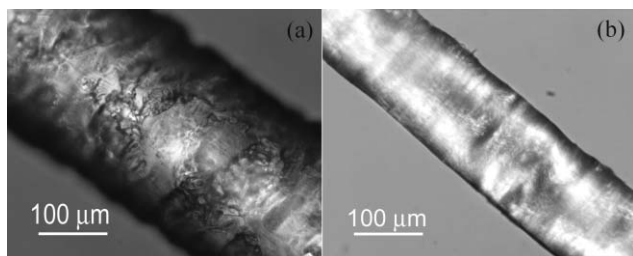
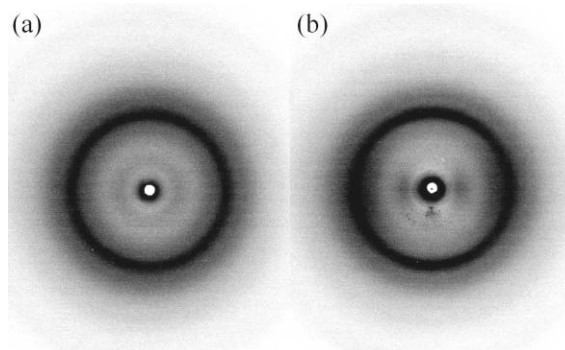
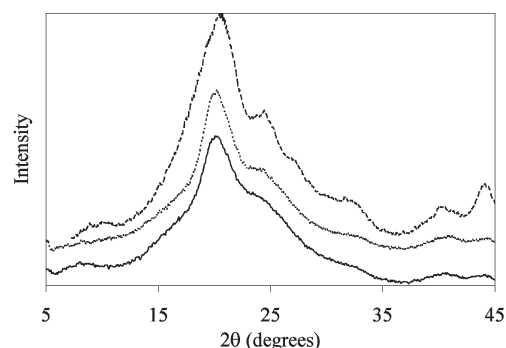
<sup>a</sup> Only a small amount of silk remained in a fiber skeleton. <sup>b</sup> The ionic liquid solution was immiscible with the coagulation solvent.

Table 1. The methanol bath formed clear, solid fibers, the acetonitrile bath formed crusted, white fibers, and the water bath appeared to form fibers similar to the methanol bath. The remaining baths of ethyl acetate, acetone, and hexanes all resulted in rapid droplet formation from the extruded spin dope; these droplets were immiscible with the bath solvents and did not coagulate. In cases where fibers persisted, the fibers were allowed to remain in their respective coagulation baths overnight to leech the residual EMIC.

For the methanol, acetonitrile, and water coagulation baths, only the fibers from the methanol bath could be handled. Sections of the methanol bath fibers were hand drawn to twice ( $2\times$ ) their as-spun length. Both the as-spun and  $2\times$  methanol rinsed fibers were allowed to air-dry to remove the bath solvent. The acetonitrile bath fibers adhered to the bottom of the coagulation bath tank and fractured during attempts to remove them. The water bath fibers broke and collapsed when removed from the bath.

The diameters of the undrawn and  $2\times$  draw ratio methanol bath fibers were 250 and 150  $\mu\text{m}$ , respectively. Both groups of samples were brittle when dried. Fig. 1 shows polarized optical micrographs of the undrawn and  $2\times$  draw ratio fibers. Both fiber samples were crystalline, however, the crystallites appeared to be aligned in the  $2\times$  sample.

The crystallite alignment of the fibers was further examined by wide angle X-ray scattering (WAXS). As shown in Fig. 2, the WAXS data for the as-spun fiber do not show significant alignment. In contrast, the WAXS data for the  $2\times$  draw ratio fiber appear to show crystallite alignment along the fiber axis. Trabbic and Yager showed that the orientation in their fibers plateaued at a draw ratio of  $3\times$ ,<sup>3</sup> while Um *et al.* showed that their crystalline orientation was still increasing at a draw ratio of  $5\times$ .<sup>8</sup> Both showed that the onset of crystallite alignment is prevalent at a draw ratio of  $2\times$ .

**Fig. 1** Polarized optical microscopy images of silk fibers coagulated in methanol (a) as spun and (b) with a  $2\times$  draw ratio.**Fig. 2** WAXS data for silk fibers coagulated in methanol (a) as spun and (b) with a  $2\times$  draw ratio. The fibers were positioned vertically in the images. The drawing process induced alignment of the crystallites.**Fig. 3** Radial intensity scans of the WAXS data for the methanol coagulated fibers for an as-spun fiber (bottom), a  $2\times$  draw ratio fiber (middle), and a natural cocoon fiber (top).

Radial intensity WAXS data for the as-spun and  $2\times$  draw ratio fibers indicate that both fibers have the same crystal structure. Fig. 3 shows the data for the as spun,  $2\times$  draw ratio, and sericin stripped, natural cocoon fibers. Both the regenerated fibers and the cocoon fiber have peaks at  $2\theta$  of  $21^\circ$  and  $24^\circ$ , correlating to 0.45 and 0.37 nm  $d$ -spacing, respectively. These peaks correlate to the  $\beta$ -sheet, Silk II structure of the natural cocoon silk.<sup>14,15</sup>

Of the six coagulation bath solvents tested, only methanol produced fibers that could be handled. The polarized optical microscopy and WAXS data indicated that crystallization took place during the fiber spinning process. Methanol is known to induce  $\beta$ -sheet formation in silkworm silk<sup>7,9,16</sup> and is also able to dissolve EMIC. Therefore, the methanol treatment may be primarily responsible for the initial crystallinity in these fibers. Subsequent fiber drawing was required to align the crystallites along the fiber axis.

Although water and acetonitrile coagulation baths did not produce any fibers that could be harvested, they still may have some potential use, especially in binary coagulation baths. Previous work showed that water used as a coagulation bath for silk films cast from ionic liquids resulted in complete dissolution of the film.<sup>13</sup> It is interesting to note here that a small portion of the silk remained as an insoluble fiber for the water coagulation bath. The only difference in processing was the shear present during the extrusion. This most likely occurred for the acetonitrile bath, but

the evidence was masked by the precipitated, amorphous silk. Ongoing research with a more sophisticated fiber-spinning apparatus using ionic liquid spin dopes may indeed prove water and acetonitrile to be reasonable coagulation bath solvents. Recently, it has been shown that silk from the spider *Nephila clavipes* exhibited increased crystalline alignment when the silk was spun while the animal's abdomen was immersed in a water bath.<sup>17</sup> That work demonstrated that stiffness and toughness of the resulting silk extends beyond what occurs in the spinneret.

Given the success of spinning silkworm silk from ionic liquid spin dopes, it may be reasonable to spin other fibrous materials. Swatlowski *et al.* demonstrated that cellulose is soluble in BMIC.<sup>18</sup> Using water as a wash solvent, they were able to form cellulose films from their solutions. Extruding these cellulose solutions through a spinneret should produce cellulose fibers. Spider silk from the dragline of *Nephila clavipes* is also a candidate for wet spinning from ionic liquid solutions. Seidel *et al.* have shown that *N. clavipes* dragline silk dissolved in HFIP can be used as a spin dope to extrude fibers with a wet spinning process.<sup>19</sup> The hydrogen bond disruption capability of ionic liquids should allow for *N. clavipes* spin dopes.

The preliminary work presented here demonstrates the feasibility of spinning fibers from *B. mori* fibroin solubilized in ionic liquids, such as EMIC. The effect of the coagulation bath solvent indicated that methanol worked best for solidifying the fibers. The methanol bath induced  $\beta$ -sheet crystallite formation, however, post-spin drawing was required to induce crystallite alignment. Acetonitrile and water coagulation baths also worked to a limited degree, but the resulting fiber properties were not desirable. These initial data do not eliminate the possibility of using acetonitrile and water as coagulation bath solvents, as shear in the spinneret could induce  $\beta$ -sheet crystallite formation, leading to fibers with more desirable properties.†

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† The EMIC for this work was prepared by the procedure outlined by Fox *et al.*<sup>20</sup>

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