Published in final edited form as:

Biofabrication. ; 9(2): 025025. doi:10.1088/1758-5090/aa711b.

Introducing Biomimetic Shear and Ion Gradients to Microfluidic Spinning Improves Silk Fiber Strength

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Abstract

Silkworm silk is an attractive biopolymer for biomedical applications due to its high mechanical strength and biocompatibility; as a result, there is increasing interest in scalable devices to spin silk and recombinant silk so as to improve and customize their properties for diverse biomedical purposes.^[1] While artificial spinning of regenerated silk fibroins adds tunability to properties such as degradation rate and surface functionalization, the resulting fibers do not yet approach the mechanical strength of native silkworm silk. These drawbacks reduce the applicability and attractiveness of artificial silk.^[2] Here, we used computational fluid dynamic simulations to incorporate shear in tandem with biomimetic ion gradients by coupling a modular novel glass microfluidic device to our previous co-axial flow device. Fibers spun with this combined apparatus demonstrated a significant increase in mechanical strength compared to fibers spun with the basic apparatus alone, with a three-fold increase in Young's modulus and extensibility and a twelve-fold increase in toughness. These results thus demonstrate the critical importance of ionic milieu and shear stress in spinning strong fibers from solubilized silk fibroin.

1. Introduction

The domesticated silkworm, *Bombyx mori* (*B. mori*), spins the silk protein fibroin into fibers with extraordinary mechanical properties.^[1] With a high tensile strength of 650 MPa, breaking strain of 12% and Young's modulus of 5.1 GPa, silkworm silk is as strong as many formulations of steel.^{[3][4]} Processes for artificially spinning silk open up the possibility of customizing mechanical and chemical features of silk fibers for a wide range of biomedical uses. As examples, silk fibers can be spun to have specific mechanical properties and degradation times tailored for use in sutures, drug delivery depots, and tissue scaffolds.^[1] Furthermore, artificial fibroin proteins with tailored mechanical and chemical properties can be genetically engineered; after bacterial expression, these proteins can then be artificially

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Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

spun into fibers. Functional artificial silk spinning is thus a technological advancement well worth pursuing.^[5]

However, current artificial spinning techniques, such as wet spinning, electrospinning, and microfluidic processing, have yet to produce silk fibers that recapitulate the mechanical strength of native silk, which has limited their usefulness. Many of these techniques also have significant drawbacks. For example, wet spinning often uses harsh chemicals that can have adverse effects on the body, while electrospinning generates an unordered mesh that is unsuitable for single-fiber applications such as sutures.^[2]

By comparison, microfluidic processing has significant advantages, since it uses biocompatible solvents and allows for the greatest control of the silk spinning process. More significantly, it offers the potential for mimicry of the arthropod silk spinning process, which would allow it to take advantage of naturally evolved efficiency. In microfluidic processing, dissolved fibroin is flowed through a micron-sized channel flanked by acidic solutions, mimicking the pH gradient during transit down a silk gland.^[2] However, this process does not currently mimic the shear, metal ions, and active transport found in the natural silk spinning process, which all play critical roles in controlling the mechanical integrity of spun silk.

Shear is important in the natural spinning process with the fibroin solution, or silk dope, because it complements the mechanical alignment induced by elongation.^[6] The tapering of the anterior division of *B. mori* silk glands, from a diameter of 400 μm at its origin down to 50 μm at the spigot, exposes the silk dope to significant shear.^{[7][8]} Rheological and confocal microscopy experiments have shown that native fibroin solutions undergo structural changes in response to shear, resulting in increased crystallinity and shear-induced gelation.^{[9][10]} More detailed studies suggest that shear could be important in silk spinning by altering the molecular alignment to increase the β -sheet content of the nascent fiber, which is known to improve its mechanical properties.^[2]

Ionic composition of the silk dope is also important for the structural strength of the resultant fiber. Using spectrometric and rheometric methods, addition of K^+ , Ca^{2+} and H^+ to silk fibroin solutions have been shown to alter their viscosity and fibroin conformation.^{[11][12]} Moreover, these ions have opposite effects; Ca^{2+} increases fibroin solubility, while K^+ and H^+ induce fibroin aggregation and precipitation, suggesting an ability to fine-tune the solubility state of the silk solution.^[13]

As changes in solubility state and shear profile can affect the structure of resultant silk fibers, control of these factors is pivotal in increasing artificial silk fiber strength and custom-tailoring its mechanical properties. The goal of the present study is to incorporate biomimetic shear and ions in modeling and fabrication of a silk-spinning microfluidic device, and to subsequently examine their impact on resulting silk fiber properties. Our use of computational modeling software in an iterative workflow of design, implementation and testing provides a powerful launch pad for future development of microfluidic spinning devices. The modularity of the designs makes it possible to probe effects of ion gradients and shear on silk spinning both independently and in tandem. Our results show that silk spun

with the incorporation of both shear stress and ion gradients have synergistic improvements in control and predictability of fiber outcomes, which are key goals of this study. Furthermore, the simplicity and low cost of this approach also allows for easier scale-up to larger-scale production.

2. Experimental Section

2.1. Materials

Polyethylene oxide (50,000 MW, Sigma Aldrich, St. Louis, MO) was dissolved in dH₂O at 3% w/v. Regenerated silk fibroin solution was prepared from *B. mori* cocoons using a previously published protocol to form a ~8.3% w/v concentration.^[29] Ethyl-2-cyanoacrylate (common name Loctite) was used as an instant adhesive to secure silk fibers.

2.2. Device Design by Computational Modeling

Commercial software FLUENT 14.0 (ANSYS Inc., Canonsburg, PA) was used to calculate numerical solutions to the multiphase flow of complex geometries and to test microfluidic designs for similarity to the silk gland in shape, diameter, shear rate and ion transport. A 3-D model of our preexisting device was constructed using channel dimensions described previously. Briefly, our preexisting device consists of a center silk inlet channel (400 μm x 1600 μm x 60 μm) and two side PEO inlet channels (400 μm x 1600 μm x 110 μm) connecting to a single outlet channel (400 μm x 5000 μm x 160 μm) in a cross geometry. The channels are oriented such that the silk inlet channel and the outlet channel are aligned along the Z-axis while one PEO inlet channel is displaced +25 μm and the other PEO inlet channel is displaced -25 μm in the Z-direction. The asymmetrical displacement of the PEO inlet channels facilitates hydrodynamic focusing from all directions to minimize clogging due to adsorption of proteins to the channel walls and maximize the interface between the silk and acidic PEO (figure 1a).^[2] Meshing was performed so that the maximum face size and maximum length were both 20 μm. Fluid dynamics of multiphase flow were simulated using the volume of fluid method (VOF), which allows for time-dependent calculations.^[30]

To determine the relationship of the PEO to silk velocity and the change in resultant fiber diameter, PEO velocity was varied from 1 mm/s to 6 mm/s. The diameter of the silk stream at the end was adjusted to match the diameter of the silk after drying with the following relationship (Equation 1), where the concentration in w/v and density of silk, were taken to be 0.083 g/cm³ and 1.4 g/cm³, respectively.^[31]

$$dry\ diameter = \sqrt{\frac{(wet\ diameter)^2 * concentration\ in\ w/v}{density\ of\ silk}} \quad (1)$$

To accommodate the effect of electric potential on diffusion for each ion species, ion transport was modeled using the coupled Nernst-Planck and Poisson equation system for electrodiffusion (Equation 2 and 3), where c_i is the concentration of species i , D_i^* is the effective diffusion coefficient for species i , z_i is the valency of species i , e is the elementary

charge, k_B is the Boltzmann constant, T is the temperature, and φ is the electrostatic potential.^[32]

$$\frac{\partial c_i}{\partial t} = \frac{\partial}{\partial x} \left[D^*_i \left(\frac{\partial c_i}{\partial x} + \frac{z_i e}{k_B T} \frac{\partial \varphi}{\partial x} c_i \right) \right] \quad (2)$$

$$\frac{\partial}{\partial x} \left(\frac{\partial \varphi}{\partial x} \right) + \sum_i z_i c_i = 0 \quad (3)$$

As diffusion is hindered in polymer solutions, the Mackie-Meares model (Equation 4) was used to predict an effective diffusion coefficient (D^*) from the volume fraction (Φ) of both fibroin and PEO solutions.^[33] Diffusion coefficients for H^+ , K^+ , Ca^{2+} , and Cl^- are $9.31 \times 10^{-9} \text{ m}^2\text{s}^{-1}$, $1.957 \times 10^{-9} \text{ m}^2\text{s}^{-1}$, $7.92 \times 10^{-8} \text{ m}^2\text{s}^{-1}$, and $2.032 \times 10^{-9} \text{ m}^2\text{s}^{-1}$ respectively.

$$D^* = \frac{1-\Phi}{(1+\Phi)^2} D \quad (4)$$

Models of the novel designs were drafted in FLUENT. The diameter and length parameters of the pulled glass micropipette for the shear device were taken from both literature values and from images taken of the micropipette tip with brightfield microscopy.^[34] The shear device has an outer diameter of $1000 \mu\text{m}$ and an inner diameter of $580 \mu\text{m}$ at its base and tapers for $3000 \mu\text{m}$ down to an outer diameter of $70 \mu\text{m}$ and an inner diameter of $50 \mu\text{m}$ at its tip (figure 1c).

The shear and metal ions device were then designed to function in tandem, with the pulled micropipette tip replacing the input channel of the sheath flow device to generate the modular composite device. A 3D model was constructed by importing the individual module models and in-model replacement of the channel by the pulled micropipette tip. The pulled micropipette tip was positioned such that it penetrated $1750 \mu\text{m}$ into the output channel of the sheath flow device (figure 1e). To eliminate discontinuities in the simulation, sharp edges in the resulting model were rounded to have radii of $10\text{--}20 \mu\text{m}$ based on size.

Meshing and simulation parameters ensured effective comparison between devices. In addition, velocities of silk and PEO for each device were calculated such that volume flux was constant for comparability.

2.3. Sheath Flow Device Generation

The sheath flow device was produced using lithographic techniques as previously described.^[35] Briefly, a photoresist-coated silicon wafer was exposed to UV light through a patterned optical mask, generating a master mold which was used to cast polydimethylsiloxane (PDMS). Two PDMS casts were subjected to air plasma and sealed

together. Holes were punched, and then the apparatus was attached to a custom-built syringe pump for spinning. Flow speeds of 8% silk and 3% PEO were set at standard values of 0.22 mm/s and 0.62 mm/s. Silk fibers were submerged in a 70% methanol bath for dehydration and collected by hand for further analysis. These parameters have been used previously for spinning silk using this device.^[35]

2.4. Metal Ions Device Generation

Ion gradients were implemented in sheath flow by adding KCl and HCl to the PEO and CaCl₂ to the regenerated silk fibroin (RSF) solution, facilitating diffusion between the two solutions during flow through the device. The ionic silk and PEO solutions were generated by dialysis against ionic solutions of PEO (MW 10,000, Sigma Aldrich). The concentrations of KCl, CaCl₂ and HCl were derived from ion concentration measurements taken from silkworms *in vivo* (Supplemental table S2).^{[11][12][13]} Computational simulations iteratively tested the concentrations and spinning velocities to establish the correct ion gradients within the device.

2.5. Shear Device Generation

Borosilicate glass micropipettes with an O.D. of 1 mm and an I.D. of 0.58 mm were pulled using a P-90 micropipette puller (Sutter Instrument Company, Novato, CA). The tip was then broken to generate a 50 μ m ID. The unpulled end of the micropipette was inserted into a 16 G x1" syringe needle, and polyethylene tubing was inserted onto the other side of the needle. The device was then sealed with epoxy to protect against leakage.

The previously described ionic silk solution was flowed through the polyethylene tubing using a syringe pump at an input velocity of 25.8 μ m/s, determined by equating the volume flux of silk (this is determined using the same volume flux of silk as that described in Section 3.1.2).^[15]

2.6. Shear To Ions in Tandem (STIT) Device Generation

To implement shear flow and ion gradients in a single device, a pulled glass micropipette replaced the silk input channel in a sheath flow device. A sheath flow device was sectioned so that the silk inlet channel was removed, and the output channel was exposed. A pulled glass micropipette was then placed in the device, which was sealed shut with epoxy. The 8% silk and 3% PEO flowed through the channels at average velocities of 25.8 mm/s and 0.62 mm/s respectively. Ion concentrations in both solutions were chosen based on values in Supplemental Table 2.

2.7. Fiber Spinning

Fibers were moved into a methanol bath after spinning, taking care not to stretch the fiber beyond its original length. Afterwards, the fibers were incubated briefly in dH₂O before being dried completely in air and analyzed.

2.8. Brightfield Microscopy

Silk fibers collected from the experimental device were observed via brightfield microscopy at 50x magnification using an Axiovert S25 inverted microscope (Carl Zeiss Inc.,

Thornwood, NY). Three separate fibers were measured for each test condition. Three pictures were taken of each fiber and five diameter measurements were taken along the fiber in each picture by image analysis in ImageJ (National Institutes of Health, Bethesda, MD).

2.9. Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy with a Nicolet 4700 Fourier Transform Infrared Spectrometer (Thermo Fisher Scientific, Waltham, MA) was used to test whether fibroin secondary structure was affected by any of the experimental conditions. Fibers were collected as previously stated, rolled into a small ball and pressed into a flat disc for measurement. A total of 256 scans were collected per disc and the spectra from each scan were added to create the final spectrum with a resolution of 4 cm^{-1} . A negative control spectrum of a blank stage was subtracted for normalization purposes. RSF silk fibers obtained from the sheath flow device without ion gradients was collected for comparison. Previously generated IR spectra of native *B. mori* silk fibers were also included in the comparison.

The β -sheet content of each fiber type was quantified through Fourier self-deconvolution (FSD) of the amide I^[16] and amide III^[17] bands, as these peaks are characteristic of β -sheet secondary structure. This technique was adapted from a previous study.^[16] Briefly, a linear baseline was subtracted from the region of interest, and a nine-point Savitsky Golay smoothing filter was used to determine the smoothed second derivative of the spectra, from which peaks were determined. Apodization with a Blackman-Harris function was performed automatically. Peaks were curve-fit from Lorentzian line shapes with an initial HWHM of 4 cm^{-1} . β -sheet content was determined by assigning peaks from literature values and normalizing the area of β -sheet peaks by the total band area.^[16] For visual comparison, the 1620 cm^{-1} peak was normalized to its maximum value, and the 1260 cm^{-1} peak was normalized to the peak at 1220 cm^{-1} , as previously reported.^{[17][18]}

2.10. Mechanical Testing

Mechanical testing was performed using a custom-built uniaxial tensile tester.^{[36][37]} Briefly, silk mechanical testing frame designs were drafted in Microsoft PowerPoint and printed onto transparencies. Instant adhesive (ethyl-2-cyanoacrylate) was added to both sides of the frame. Spun silk fibers were laid across the frame after a brief immersion in distilled water to aid in hydration. Two polyoxymethylene squares were loaded on top of the adhesive, securing the silk fiber. The frame was then screwed onto the mechanical force sensor to prevent movement during the pulling process. A soldering iron was used to melt the sides of the plastic frame so that only the silk fiber would be subjected to mechanical force, and the mechanical force sensor pulled the fiber lengthwise at a strain rate of 0.01 s^{-1} until fiber failure. The strain rate was chosen to match that of similar mechanical tests for previous tests of silk fibroin fibers artificially spun from microfluidic devices.^[2] For each test condition, ten replicate fibers were tested. Fibers that failed before the mechanical stretch were not included in data analysis.

Data analysis was performed on both raw data and fiber diameter measurements obtained from brightfield microscopy to generate a stress-strain curve for each fiber pulled. A rolling

average was performed on the data to smooth the curves. The Young's modulus was determined by taking the highest slope of the stress-strain curve. The elongation was determined by taking the difference in strain between the point of initial stretching and the point of total failure. The toughness was determined by integrating the stress-strain curve over the interval of elongation. These values were then compared to each other and to published values of native *B. mori* silk fibers.^[4]

3. Results

3.1. Modeling and Generation of Devices

Our previous work has described a simple sheath flow microfluidic device (referred to as sheath flow device) capable of spinning silk from a silk fibroin solution using the principle of flow-focusing.^[2] The silk fibroin solution is forced through a linear channel which becomes flanked by flow from two channels of polyethyleneoxide (PEO). The acidic PEO lowers the pH of the silk stream, inducing polymerization of the fibroin into silk fiber as it exits the device. We used ANSYS FLUENT to design 3D models of similar microfluidic devices incorporating biomimetic metal ion gradients and shear forces. In these simulations K⁺ and H⁺ ions were introduced into the PEO to create inward K⁺ and H⁺ gradients and an outward Ca²⁺ gradient from the silk stream (referred to as metal ions device). Shear was generated by forcing fibroin through a tapered pulled-glass pipet mimicking the geometry of the silkworm silk gland (referred to as shear device). Experimental and literature-derived values were used to construct geometries of the sheath flow, shear, and integrated modular devices. To predict the flow profile, spinning parameters were implemented on these geometries in a volume of fluid (VOF) formulation for multiphase flows. A 0.1 s interval was chosen as an optimum time step for fast and accurate calculations, and simulations were run for 45 s total. The model assumed no slip conditions for the wall and no gravity effects with constant input velocity and ambient output pressure. The solutions used for these calculations were 8% w/v regenerated silk fibroin (RSF) with a viscosity of 0.02 Pa*s, and 3% w/v PEO solution with a viscosity of 0.4 Pa*s.^[14] Constant viscosity was modeled for these solutions for ease of simulation. The flow of silk and PEO solutions was simulated with average velocities of 0.22 mm/s and 0.62 mm/s, respectively.

Elongational flow and shear stress simulations were run for all constructed devices (figure 1). Computational simulations of flow within the sheath flow device successfully predicted elongational flow of silk solution within the device as the silk solution comes into contact with adjacent PEO solutions. The shear device was predicted to generate wall shear stresses of up to ~12 Pa. Silk flowing through the sheath flow device was predicted to experience shear rates of $<10 \text{ s}^{-1}$, while silk flowing through the shear device and the integrated 'Shear To Ions in Tandem' device (referred to as STIT device) was predicted to experience shear rates of up to 473 s^{-1} , comparable to native silk which is predicted to experience shear rates of up to 510 s^{-1} (figure 2).

The computational model was also used to predict silk fiber diameter as a function of varying spinning velocities. These predictions were then validated against experimental diameter measurements (figure 3). The results show that the computational models were able to successfully predict fiber diameters from the sheath flow device.

3.2. Effects on Fiber Spinning

After several computational iterations, microfluidic devices were fabricated to finalized design specifications via photolithographic, soft lithographic, and micropipette pulling procedures. These were then tested for silk fiber production. Several devices were constructed and tested: the sheath flow device, the shear device, the metal ions device, and a device incorporating both shear and ion gradients in sheath flow. This 'Shear To Ions in Tandem' device (STIT device) comprised of insertion of the outlet of the shear device into the inlet of the metal ions device.

The sheath flow, metal ions, and STIT devices successfully produced silk fibers. In contrast, as predicted by the computational model, the shear device was incapable of producing fibers, likely stemming from shear stresses being significantly lower in the device than the natural gland despite similar shear rates.^[15] However, its use as one of the modules in the STIT device improved the quality of the silk produced, as we show below.

3.3. Effects on β -sheet Content

β -sheet content of silk fibers is a major determinant of fiber strength. The fibers spun from the devices were therefore investigated via ATR-FTIR. Total FTIR spectra of spun fibers were qualitatively assessed against the published spectrum of native silk fiber (Supplemental figure 1). Silk β -sheet content was then investigated by comparison of changes to characteristic peaks, specifically increased absorbance at the 1260 cm^{-1} peak and a shift in the 1620 cm^{-1} peak to lower wavenumbers.^{[16][17][18]} These peaks were isolated for deeper analysis (figure 4). Fourier self-deconvolution of these peaks was also performed to estimate relative β -sheet content. The addition of ion gradients in the metal ion device caused shifts in both FTIR peaks (figure 3A), with the 1260 cm^{-1} peak increasing in absorbance compared to the sheath flow device, and the 1620 cm^{-1} peak shifting to lower wavenumbers as would be expected from an increase β -sheet content. Deconvolution of the amide I band (figure 3B) gave further evidence of this increase, showing that the spun silk's β -sheet content had a relative increase from 32% to 37%.

Fibers spun from the STIT device also showed these characteristic shifts, suggesting increased β -sheet formation compared to the sheath flow device. Deconvolution of the amide I band gave a calculated β -sheet content of 36%, which, although increased compared to sheath flow device, is not increased compared to the metal ions device. These results suggest that addition of metal ions increases β -sheet content, while addition of shear does not.

3.4. Effects on Mechanical Strength

The mechanical properties of silk spun from the devices were next investigated via tensile testing. The tensile testing data were analyzed for quantitative mechanical properties including Young's modulus, extensibility, toughness, and ultimate tensile strength (figures 5 and 6). Silk fibers spun from the metal ions device showed significant increases in all measures of mechanical strength compared to fibers from the sheath flow device, evidencing the positive effect of the addition of ions. Fibers from the STIT device had even greater extensibility and toughness (Supplemental table S1), suggesting that shear also contributes to the mechanical strength of spun silk.

3. Discussion

We created a novel modeling system for microfluidic processing, and used the model to aid in the design of novel microfluidic devices. Our models successfully predicted the relationship between the diameter of the silk thread and the flow rate of the fibroin solution in the constructed devices, and also led to the successful prediction that the shear device would be unable to spin silk. The latter prediction was based on the calculated shear stress at the tip of the pipette; at ~12 Pa, the shear stress generated by the device was three orders of magnitude lower than the ~20 kPa experienced naturally in the *B. Mori* silk gland.^[15] In contrast, geometrical differences between the device and native *B. Mori* silk gland were unlikely to play a significant role.^[15]

The lower shear stress in all devices compared to native glands is also partially responsible for the lower β -sheet content of spun fibers (32–37% compared to 54% in native silk fibers). Lower shear stress is likely due in part to the 8% fibroin concentration of RSF solutions used in our devices as opposed to 30% in native silk dope.^[12] To address these differences, shear stress could be increased in future studies by using more concentrated silk solutions. Increasing the extrusion rate is also known to increase shear, and can be accomplished by pulling the silk as it extrudes from the microfluidic device. This process closely mimics the native process of repeatedly anchoring and pulling the silk fiber from the gland.^[19] These two methods have been shown to increase the mechanical properties and β -sheet content of fibers spun using other techniques to be closer to native values, and may further build on the increases observed in this paper to produce silk of even greater quality.^[20]

Our experimental results showed that complete fibers were successfully extruded from the STIT device, both with and without implementation of ion gradients. This finding was contrary to our model, which predicted failure of fiber formation from the device, as the silk solution streaming from the embedded micropipette tip was calculated to spray non-directionally upon entering the lower pressure environment of the sheath flow component. The discrepancy between computational and experimental results suggests the presence of variables currently unaddressed by the computational model, perhaps related to more complex effects of shear, or the interaction of shear with ions on fibroin assembly. One possibility is that having the shear device lead into the sheath flow device mitigates the issue of small diameters mentioned above. The flowing solvent allows for the encapsulation of the silk stream in the center of the sheath flow, limiting turbulence.

Fibers spun by the STIT device have superior mechanical properties to those spun by metal ion device; however, they do not show a concomitant increase in β -sheet content, despite evidence that shear induces alignment of fibroin molecules and contributes to β -sheet formation. Shear rates within the shear device and the STIT device (up to 473 s^{-1}) closely matched previously predicted shear rates within native *B. mori* silk glands (up to 510 s^{-1}). As such, silk spun from these devices were expected to have increased β -sheet content relative to silk spun from the sheath flow device, given the latter device's significantly lower shear rates ($<10 \text{ s}^{-1}$).^[15] Possible explanations include insufficient shear stress exerted at the pipette tip, and that shear and ion gradients are implemented in tandem instead of concurrently. As described above, the ~12 Pa shear stress exerted at the pipette tip is

significantly lower than that exerted in the native *B. Mori* silk gland, and may be insufficient to induce β -sheet alignment. Alternatively, these results could also reflect correctable deficiencies in the design of the STIT device. The impermeable pulled micropipette penetrates a significant depth into the multiphase output channel in the STIT device, giving the silk stream a shorter exposure to the surrounding PEO. This shorter transit time could potentially be insufficient for full ionic equilibrium across the PEO-RSF boundary. The transit time limitation may be resolved by increasing the length of the output channel, allowing the RSF silk stream to experience a longer exposure to the ionic gradient across the RSF-PEO boundary. An alternative approach would be to use a nanoporous pulled micropipette, which would allow ions to interface the RSF solution while still providing a wall for shear stress generation. Microfluidic designs have previously made use of nanoporous glass, but nanoporous glass has yet to be used for silk spinning.^{[21][22]}

How the addition of shear stress to ion gradients in the STIT device increases the mechanical strength of the spun fiber without an associated increase in fiber β -sheet content remains to be determined. Previous studies have shown that shearing regenerated silk fibroin solution results in higher β -sheet content from the elongation and alignment of protein globules, but have typically spun silk protein concentrations upwards of 33%, significantly higher than ours at 8%.^[8] Increased concentration results in increased shear during the spinning process, and thus increased β -sheet content, which was surmised to be the cause of increased fiber mechanical strength.^{[23][24]} Our results, however, suggest the possibility that the magnitude of shear required for increased β -sheet content may be greater than that for increasing mechanical strength.

β -sheet content is not the sole determinant of native silk's superior mechanical strength. One possibility is that shearing may cause greater alignment of silk polymers in the direction of flow, increasing polymer slippage and extensibility. As drawing is not performed before mechanical testing, undrawn fibers may have similar levels of β -sheet content while having different molecular orientations, which can be confirmed by wide-angle X-ray scattering.^[25] In addition, several important non- β -sheet secondary structure motifs have been found to contribute to the mechanical strength of spider silks, such as 310-helix forming repeats GXX and elastic -spiral regions composed of multiple GPGXX motifs.^{[26][27][28]} It is possible that structurally or functionally analogous motifs are present in silkworm silk. Further investigation into these possibilities may help shed light on the roles and interplay between shear and ion gradients for silk spinning by arthropods, and for use in device design.

4. Conclusion

In this study, we have added the biomimetic aspects of ion gradients and shear stress to current microfluidic methods of silk processing, improving the quality of silk fibers produced. Ion gradients in coaxial flow improved the mechanical properties of spun silk compared to previous designs, while as predicted, shear as produced by a pulled micropipette device did not produce fibers. Tandem integration of both shear and ion gradients in the STIT device resulted in spun fibers with clear mechanical superiority compared to either alone, causing an increase in β -sheet content from 31% to 36%, a threefold increase in Young's modulus and extensibility, and a twelvefold increase in

toughness. Modularity of the components allows examination of both aspects individually and in tandem, and the simplicity of fabrication suggests that scale-up could be easy and low-cost. The iterative workflow of design, modeling, and experimentation makes the system readily modifiable for optimization. These improvements are a major step forwards toward the goal of strong, tunable artificial silk fibers for use in surgical threads, drug delivery depots, and tissue engineering scaffolds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH U01 EB014976 (J.Y.W. and D.L.K.), and the ED Graduate Assistance in Areas of National Need Fellowship (D.L.). The authors would like to acknowledge Dr. Xin Brown from the Boston University BioInterface Technologies Core Facility and Dr. Ze'ev Feit from the Boston University Micro/Nano Biosystems Fabrication Facilities for training assistance, Dr. Stanley Heydrick for his expert opinion and aid in the editorial process, and Yang Liu for editing the manuscript.

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Figure 1.

Representative computational fluid dynamics simulations of microfluidic devices in ANSYS FLUENT. Geometric models of microfluidic devices were constructed using experimental and literature values (a,c,e). Spinning parameters were used to simulate flow conditions to predict aspects of microfluidic silk spinning (b,d,f). Other aspects than those shown were also used to validate the models, including estimations of fiber diameter and ion transport. The interior geometry of the sheath flow device was simulated (a) and used to predict the volume fraction of silk within the channel during spinning after 45 s of flow (b). Simulations successfully predicted that silk undergoes elongational flow within the device (b, inset). The tapered end of the shear device was also simulated (c) and used to predict wall shear stress during spinning after 45 s of flow, which increases to a maximum of ~12 Pa towards the tip (d). Finally, the STIT device was simulated (e) and used to predict the volume fraction of silk solution after 45 s of flow (f). The model predicted that upon leaving the micropipette tip, the silk would spray non-directionally into the outlet channel (f, inset), preventing fiber assembly. The sheath flow device (a) consists of a silk input channel (green, 400 μm x 1600 μm x 60 μm), two PEO input channels (blue, 400 μm x 1600 μm x 110 μm), and an output channel (red, 400 μm x 5000 μm x 160 μm). As shown, the two PEO channels are asymmetrically positioned. The shear device (c) has an outer diameter of 1000 μm and an inner diameter of 580 μm at its base and tapers for 3000 μm down to an outer diameter of 70 μm and an inner diameter of 50 μm at its tip. The STIT device combines the sheath flow and shear device geometries. Scale bars are 1 mm (a–b,e–f) and 500 μm (c–d).

Figure 2.

Shear rate prediction. Silk spinning with the sheath flow device (a), the shear device (b), and the STIT device (c) was simulated in ANSYS FLUENT. Shear rates in the middle of the device is shown. As silk only flows through the center of the channel, it experiences shear rates of $<10 \text{ s}^{-1}$ in the sheath flow device while experiencing shear rates up to 473 s^{-1} in the shear device and the STIT device. The maximal shear rate and shear rate profile are similarly located in both the shear device and the STIT device (b,c, inset), while relatively minimal shear is present in the rest of the STIT device. Scale bars are 1 mm (a,c) and $500 \text{ }\mu\text{m}$ (b).

Figure 3.

Fiber diameter prediction. Silk spinning with the sheath flow device was both simulated and experimentally performed, with the silk velocity held constant at 0.22 mm/s. Predicted fiber diameters were found to closely match experimentally measured diameters. Computational predictions of fiber diameter as a function of PEO average velocities were made using ANSYS Fluent (a). Image analysis was used to determine the cross-sectional areas of the silk stream in the device, and the dehydrated silk fiber cross-sectional areas were predicted by multiplying the cross-sectional area by the volume fraction of silk in the solution. The analysis was repeated for five simulation runs. Fibers were then spun using the set PEO velocities, and the diameters of these fibers taken via analysis of brightfield microscopy images in ImageJ (a, inset). Three fibers were used for each PEO velocity, with three images taken of each fiber; diameters for each image was taken at five distinct points. The predicted and experimental values were in close agreement, demonstrating the accuracy of the multiphase model used (b). Scale bars are 10 μm (a) and 30 μm (a, inset). Error bars indicate standard error.

Figure 4.

Effects of metal ion gradients and the combination of metal ions and shear on silk fiber FTIR spectra. FTIR spectra of the silk spun with the metal ions device (squares) showed both an increase in peak at 1260 cm^{-1} (a, arrow) and a shift to lower wavenumbers in the peak at 1620 cm^{-1} (b, arrow); both are characteristic of increased β -sheet content. A further increase in the peak at 1260 cm^{-1} was observed for fibers subjected to metal ion gradients and shear in the STIT device (circles). 256 scans were performed on each sample, and the spectra from each scan were added to generate the final spectrum with a resolution of 4 cm^{-1} . (a) and (b) are from the same spectrum.

Figure 5.

Effects of metal ion gradients and the combination of metal ions and shear on silk fiber mechanical properties. Stress-strain curves are shown of silk spun from the sheath flow device (solid line, $n=9$), the metal ion device (squares, $n=6$), and the STIT device (circles, $n=4$). Ten replicate fibers were spun and briefly hydrated in water before being placed on frames and dried prior to mechanical testing. Fibers that failed before the mechanical stretch were not included in data analysis. RSF fibers spun with both metal ions and shear were tougher, stronger, and more extensible than silk fibers spun with no additional influences.

Figure 6.

Quantitative effects of metal ion gradients and combined metal ions and shear on mechanical properties. Quantification of measurements were taken from the mechanical stress-strain curves (figure 4) to confirm qualitative impressions. The extensibility (a), Young's modulus (b), toughness (c), and ultimate tensile strength (d) of fibers increased significantly after implementation of metal ion gradients in comparison to sheath flow only. The combination of ions and shear in the STIT device added further significant increases to fiber extensibility and toughness; the Young's modulus was also greater on average. Extensibility was calculated by measuring the strain interval between initial stretch and total failure. Young's modulus was calculated by taking the highest slope of the stress-strain curve over the interval, and toughness was calculated by integrating the stress-strain curve over the interval. Error bars indicate standard error.