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Development of a Process for the Spinning of Synthetic Spider Silk

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Abstract

Spider silks have unique mechanical properties but current efforts to duplicate those properties with recombinant proteins have been unsuccessful. This study was designed to develop a single process to spin fibers with excellent and consistent mechanical properties. As-spun fibers produced were brittle, but by stretching the fibers the mechanical properties were greatly improved. A water-dip or water-stretch further increased the strength and elongation of the synthetic spider silk fibers. Given the promising results of the water stretch, a mechanical double-stretch system was developed. Both a methanol/water mixture and an isopropanol/water mixture were independently used to stretch the fibers with this system. It was found that the methanol mixture produced fibers with high tensile strength while the isopropanol mixture produced fibers with high elongation.

Keywords

spider silk; spinning; mechanical properties; XRD; process development

INTRODUCTION

The Golden Orb Weaver, *Nephila clavipes*, makes six different types of silk and one glue, each with specific functions.¹ Major ampullate silk, known commonly as dragline silk, is among the strongest known natural materials.² Dragline silk has a combination of high

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomaterials.5b00092. Chart showing tensile strength of fibers produced in stretch baths containing different ratios of IPA and water in the first bath of the double-stretch system (PDF)

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tensile strength, elasticity, and extension, making it a desirable material for applications such as ropes or cords, medical sutures, synthetic tendons or ligaments, and sports materials. Studies have shown that dragline silk elicits almost no immunological response and even promotes cell growth, which makes it ideal for medical biomaterials.^{3–6}

Because of its unique properties and potential uses, dragline silk has been highly studied. Dragline silk comprises two different proteins, major ampullate spidroin 1 (MaSp1)⁷ and major ampullate spidroin 2 (MaSp2).⁸ Each of these proteins are highly repetitive and contain specific sequences which have been highly conserved.^{9,10} Both proteins contain a crystalline region that is formed by a poly alanine motif. This motif forms a crystalline β -sheet structure which is responsible for the high tensile strength of dragline silk.^{11–13} MaSp2 contains a GPGXX motif which makes up much of the presumed amorphous region of the protein and forms a β -spiral that is believed to be responsible for the elasticity of dragline silk.¹⁴ MaSp1 and MaSp2 are mixed in the dragline silk of *N. clavipes* at a ratio of 4:1,¹¹ respectively, although the ratio varies in other species.

Spiders cannot be farmed for their silk, they are cannibalistic and territorial. The most reasonable alternative for producing dragline silk fibers is to utilize genetic engineering to produce the proteins in a foreign host, then spinning the fiber in vitro. Spider silk-like proteins have been expressed in a variety of organisms including bacteria,^{15–18} Sf9 insect cells,¹⁹ and yeast,²⁰ as well as in the milk of transgenic goats.²¹

Spider silk proteins can be used to create a large variety of biomaterials, namely fibers,^{22–26} films,^{27–31} coatings,³² composites,³³ and gels.^{34–38} Various methods exist to produce each of these materials. For fibers, the synthetic spider silk protein is dissolved to form a spin dope.^{17,39} Traditionally, this has been accomplished using a chaotropic agent, though recently aqueous methods have arisen.^{18,31} This spin dope can then be extruded into a coagulation bath to form a fiber, though there are other methods and devices for creating fibers.^{40,41}

In general, fibers produced by the described method are weak and brittle unless a postspin draw is applied.^{17,22–24,26,42–44} Previous studies have shown that when a postspin draw is applied to synthetic spider silk fibers there is an increase in mechanical properties. To apply the postspin draw, the fiber is immersed in a stretch bath, which is generally aqueous isopropanol or methanol,^{17,29,30} and then stretched a defined amount. Though much progress has been made in the area of synthetic silk fibers,^{22,26,42} the process can be improved upon. Table 1 compares as-spun and post-spin-treated synthetic fiber mechanical test data. When applying a postspin draw, Teulé et al. showed an increase in tensile strength and toughness of fibers after stretching them in a mixture of water and IPA. Using the same stretch bath but with a 6X stretch instead of a 3X stretch, Heidebrecht et al.⁴⁶ showed substantial increases in mechanical properties, in particular the toughness, which was reported to be 189 MJ/m³ with low variability. Heidebrecht et al. reported several different synthetic constructs with significant increases after a postspin draw.

Substantial variability in the mechanical properties of stretched synthetic spider silk has been reported in various studies. For Teulé et al., Heidebrecht et al., and An et al., the

standard deviations for tensile strength were 10, 16, and 32% respectively. The standard deviations for strain values from the same studies were even higher, at 74, 23, and 179% of the average. The variability seen in most synthetic fibers could come from a variety of sources, such as an issue in protein quality, error in sample prep and testing, or from the stretching of fibers by hand. To make synthetic spider silk fibers commercially viable and possibly reduce the variability seen in most synthetic fiber properties, a mechanical system must be employed for processing these fibers. In this study, the creation of a mechanical system for processing fibers is presented that reduces variability and improves overall mechanical properties of spun fibers. This process includes the formulation of a functional spin dope and the optimization of a mechanical postspin draw.

MATERIALS AND METHODS

Purification of Silk Protein from Milk

The spider silk protein is synthesized by transgenic goats. There are two types of transgenic goats in the herd at Utah State University, goats that produce a MaSp1 protein analog and goats that produce a MaSp2 protein analog. The sequence of each protein are the natural spider silk protein sequences as they have been cloned previously.^{7,8} Both proteins are approximately 65 kDa long and contain both the repetitive region and nonrepetitive C-terminal of the protein. The protein is produced when the goats lactate and is accumulated in their milk. Milk is collected from the transgenic goats and then frozen and stored at -20°C until purification. Fat is separated from the thawed milk by a standard cream separator and the fat is discarded. The milk is combined in a 1:1 v/v ratio with 0.1 M L-arginine-HCL (Spectrum Chemical MFG. Corp., Gardena, CA) and the pH is adjusted to 9 with 2 M sodium hydroxide (Fisher Scientific, Fair Lawn, NJ). After mixing for 30 min, the milk is loaded onto a custom system of tangential flow filters comprised of a 750 kDa hollow fiber cartridge and 50 kDa hollow fiber cartridge²¹ (GE Healthcare, Westborough, MA). The milk is filtered in a continuous loop for 24 h until concentrated and the clarified whey is collected. After filtration, the silk protein is salted out by adding ammonium sulfate (Amresco, Solon, OH) to the whey to a final concentration of 1.2 M and allowed to mix for 24 h at 4°C . The protein is then serially washed and centrifuged with distilled water until the conductivity is observed to be below $20\ \mu\text{S}$ and subsequently lyophilized to dryness. SDS-PAGE is performed to confirm purity on samples using 4–20% gels (Thermo Scientific, Rockford, IL) with the manufacturer's recommended HEPES-SDS running buffer. The gel is stained with Biosafe Coomassie blue (Thermo Scientific, Rockford, IL). Western blots are also performed using antibodies specific to the C-terminus of rMaSP1 or rMaSP2. Recent publications have shown this method produces protein that is 90% pure.^{31,46}

Spin Dope Preparation

In a ratio of 80:20, synthetic MaSp1 and MaSp2 were placed in a 4 mL glass vial with a Teflon lid (Waters Associates, Milford MA) and then HFIP (HFIP; Oakwood Chemical, West Columbia, SC) and 88+% formic acid (Alfa Aesar, Ward Hill, MA) was added in a 4:1 ratio in order to make a spin dope that was 25% weight protein/volume solvent (wt/v). Vials were placed on a motorized rotator (Labnet, Edison NJ) and allowed to mix for 48 h at 7 rpm. Dopes were then placed in a clinical centrifuge (VWR International, Wehingen

Germany) and spun for 24 h at 4180 rcf after which any impurities are removed from the top of the spin dope and the dope is removed to a new vial.

Spinning Process

Using a modified DACA SpinLine (DACA Instruments, Santa Barbara, CA),^{39,47} the spin dope is loaded into a 1 mL Hamilton gastight syringe (Hamilton Company, Reno, NV) that had 10 cm of PEEK tubing (internal diameter 0.005") (SUPELCO, Bellefonte, PA) for a needle. The DACA was set to extrude the protein at a rate of 0.7 mm per minute into a 100% isopropanol (Pharmo-Products Inc., Brookfield CT) coagulation bath.

Single-Bath Stretching of Fibers

The fibers were submerged in either an isopropanol/water, methanol/water, or 2 M ammonium sulfate bath between two Godets, as seen in Figure 1. The two different Godets can be programmed to spin at different speeds in order to stretch the fibers while they are immersed in the stretch-bath placed between Godets. Fibers were stretched between the two godets at various speeds, from 1.5× up to 4×. Higher stretches were attempted but fibers broke before enough material was collected for testing. Alcohols that were used in the alcohol stretch-bath were either methanol (Pharmo-Products Inc., Brookfield CT) or isopropanol. Both alcohol stretch-baths were mixed with dDI (distilled, deionized) water, with methanol mixed at a ratio of 4:1 and the isopropanol at a ratio of 8:2. For water dipping experiments, a bath of water was placed between the winder the second set of godets with a small Teflon rod placed in the bath to keep the fibers submerged while traveling through the bath. Optimization of the system was based primarily on maximizing tensile strength.

Hand-Stretching of Fibers

Fibers that were stretched using the single-stretch system were cut into 10 cm lengths and immersed in a 10:90 IPA:water bath. Fibers segments were immersed for 1 min before being stretched to 15 cm by gently pulling on both ends of the fiber. Fibers were then removed from the bath and held at the 15 cm length until dry (usually 1 min).

Solubility Tests of Synthetic Silks

10 cm lengths of silk were placed in a Petri dish with dDI water to determine their solubility in water. Fibers were left at room temperature in the Petri dishes for one month and were observed weekly.

Multibath Stretching of Fibers

A third set of Godets was built in house that could be used in conjunction with the modified DACA SpinLine. This third set of Godets allowed for two mechanical stretches to be performed on the fibers as they were made. Figure 2 shows the modified DACA SpinLine with the third set of Godets. In the first bath, the fiber was stretched to 150% of its original length (1.5×) using the same baths as for the single-bath stretch described above. Fibers were then submerged in the second bath, dDI water, between the second and third set of Godets stretching the treated fiber another 2×. After both stretches, the fibers had a

cumulative stretch of 3×. As with the single-stretch system, optimization was done via maximizing the tensile strength of fibers.

Fiber Testing and Analysis

Commercial samples of KEVLAR®, para-aramid, polyester and nylon were obtained for mechanical tests. All fibers, synthetic spider silk and commercial synthetics, were tested using a procedure documented by Stauffer et. al.,⁴⁸ each fiber was taken and attached with liquid Super Glue to X-ray film that was cut for testing purposes. The gauge length of the fiber was 19.1 mm. The diameter of the fibers was obtained by measuring each sample nine times along the length of the sample using a Motic light microscope and supplied measuring software (Richmond, British Columbia, Canada). Fiber samples were secured to a MTS Synergy 100 (MTS Corporation, Eden Prairie, MN) test bed equipped with a custom 10 gm load cell (Transducer Techniques, Temecula, CA).²³ Samples were pulled at a rate of 5 mm/min until breaking and data accumulated at 120 Hz. All tests and measurements were performed at room temperature and the relative humidity during testing of fibers fluctuated between 20 and 26%. The recorded data was exported to Microsoft Excel and MatLab for analysis using piece-wise integration to determine energy to break.

X-ray Diffraction

Samples were taken to the Advanced Photon Source located at Argonne National Laboratory, Argonne IL, USA and X-ray fiber diffraction was performed on the BioCars 14bm-C beamline. Fibers were mounted and placed at a distance of 300 mm from the detector. Stretched fibers were placed with the stretched axis normal to the beamline. For a single image, data collection times were 60 s and ten images were taken for each sample. Background images were taken right after each sample was completed with identical parameters. Images were then processed using Fit2D software.

RESULTS AND DISCUSSION

Single-Stretch Bath System

Fibers were successfully spun from the spin dope created. The fibers that were collected from the coagulation bath (referred to as as-spun fibers) were white in color. Fibers had an average diameter of $60.6 \pm 1.9 \mu\text{m}$. Figure 3 shows a comparison of as-spun fibers with fibers stretched to 4X their original length. Stretched fibers were less opaque under the microscope and were significantly smaller than as-spun fibers, with 2×, 3×, and 4× stretched fibers having an average diameter of 43.4 ± 1.7 , 36.4 ± 0.4 , and $32.2 \pm 0.9 \mu\text{m}$, respectively.

As-spun fibers were difficult to handle as they were brittle and weak, with an average tensile strength of $32.6 \pm 7.4 \text{ MPa}$ and an average strain of $0.011 \pm 0.003 \text{ mm/mm}$. To process fibers, the second set of godets was set to 2×, 3×, and 4× to stretch the fiber. The stretch-bath between the godets was filled with an 80:20 mixture of IPA and water. Figure 4 shows the stress vs strain curves of fibers that were stretched in the described manner. All fibers had improved mechanical properties when compared to the as-spun fibers. As the stretch ratio was increased the tensile strength of the fibers increased, measuring 84.0 ± 9 , 143.2 ± 13.5 , and $174.3 \pm 20.2 \text{ MPa}$ for the 2×, 3×, and 4× fibers, respectively. The standard deviation of

the tensile strength for the mechanical stretches is improved in comparison to those reported for hand-stretched fibers. The strain of the fibers decreased with increasing stretch ratio, going from 0.56 ± 0.25 mm/mm at a 2 $\times$ stretch to 0.26 ± 0.11 mm/mm at a 3 $\times$ stretch to 0.07 ± 0.03 mm/mm at a 4 $\times$ stretch. From the data it would appear that an increase in tensile strength is connected to decreased strain. Stretching the fiber to 4 times its original length instead of 2 times its original length yielded over a 200% increase in tensile strength but decreased the strain of the 4 $\times$ fiber to 15% of that of the 2 $\times$ fiber. The toughness for the samples, that is the energy required to break the sample, also decreased with decreasing elongation.

When placed in water, the as-spun fibers dissolve. Two 10 cm lengths of 3 $\times$ stretch were placed in a Petri dish with dDI water to determine their solubility in water. After one month the fibers were intact, indicating that stretching the fibers in the alcohol/water bath changes their structure so that they are no longer soluble in water.

Natural dragline silk has the unique property of super-contracting in water, with studies reporting the silk losing between 25 and 40% of its original length.^{49–52} With this in mind, 10 cm sections of fibers of 3 $\times$ stretched fibers were cut and then dipped in dDI water for 1 min. Fibers were then removed and their length was measured. On average, the stretched fibers contracted 36% of their original length with a standard deviation of 11%. This implies that the structure of the stretched fibers is similar to natural spider silk.

Different baths were attempted in the single mechanical stretch system to explore the effects of different liquids on the fiber's mechanical properties. Three different baths were attempted: 80:20 IPA and water, 50:50 IPA and water and 2 M ammonium sulfate. These baths were chosen based on hand-stretching experiments using a variety of bath compositions. Ethanol was also attempted in the hand-stretching experiments but did not perform well. Fibers were stretched successfully in each of the three baths, though fibers in the 50:50 IPA:water bath often broke while at a 3 $\times$ stretch. As such, a 2.5 $\times$ stretch was gathered and analyzed for the 50:50 bath. The 2 M ammonium sulfate bath would leave the fibers with salt residue on the outside. To remove residue, the fiber was run through a water bath after the second set of godets before being collected on the winder. Table 2 shows the mechanical properties of fibers collected from each of these stretch-bath experiments. The 50:50 bath shows a large decrease in tensile strength when compared to the 80:20 bath, but has significantly improved strain, with the average sample being pulled to 143% of its original length before breaking. This confirms what other researchers have found using NMR and XRD, that with increasing water concentration comes increased mobility of the protein in the fiber.²² The 2 M ammonium sulfate fibers had nearly identical tensile strength values as the 80:20 IPA:water stretched fibers at 145.45 ± 15.99 MPa as compared to 143.16 ± 13.46 MPa of the 80:20 IPA:water bath. However, the strain of these fibers was better, averaging 0.553 mm/mm as compared to the 0.258 of the 80:20 samples.

Post Single-Stretch Treatments

Teulé et. al demonstrated that dipping hand-drawn fibers in water after being drawn and dried improved tensile strength and strain, they recorded a 2.8 fold increase in strain.²² It was theorized that the higher strain observed in the 2 M ammonium sulfate stretched

samples was potentially a result of being washed in a water bath prior to collection rather than a consequence of the stretch-bath. To determine this, we decided to do a water dip experiment using fibers that were spun using the 80:20 IPA:water stretch-bath and stretched to 3× their original length. A water bath was placed before the winder to dip the fibers before collection, just as they had been for the 2 M ammonium sulfate bath. Furthermore, it was theorized that stretching the sample a second time in water, rather than simply dipping it in water, could increase mechanical properties. To test our two-stretch theory, we collected fibers that were stretched to 3× their original length in the 80:20 IPA:water bath. These fibers were cut into 10 cm segments and then placed in a 10:90 IPA:water bath. Fibers were allowed to soak for 1 min and then fibers were pulled by hand from both sides to a length of 15 cm, meaning they were stretched to 1.5 times their original length. The results from these two experiments can be seen in Figure 5.

The 80:20 IPA:water stretched fibers dipped in water with no stretch before being collected had a tensile strength of 141.1 ± 19.5 MPa, which was statistically identical to the tensile strength of fibers that were not dipped in water as well as to the 2 M ammonium sulfate stretched fibers (p -value <0.05). However, the strain for these fibers increased from 0.26 ± 0.11 mm/mm without the water treatment to 0.66 ± 0.10 mm/mm with a water treatment, an increase of over 250%. The increase in strain over the 2 M ammonium sulfate fibers was also found to be statistically significant. With this increase in strain, the toughness also increased 215%, to 74.64 MJ/m³ with the water treatment from 34.63 MJ/m³ without the water treatment.

Fibers that were stretched a second time had an average tensile strength of 210.9 ± 15.7 MPa, significantly higher than that of the fibers stretched once. The average strain of these samples was 0.30 ± 0.09 , slightly higher than the strain of the single stretched fibers. Fibers were stretched to 1.5× the original length, this was after being stretched 3×. This means that the total stretch applied to these fibers was 4.5×. Compare the properties of these fibers to the properties of the fibers that were stretched only once, but to 4× their original length (see Figure 4). The 4× fibers had an average tensile strength of 174.3 MPa with an average strain of 0.07 mm/mm, whereas the fibers that were stretched twice to a total of 4.5× their original length had a tensile strength of 210.9 MPa and a strain of 0.30 mm/mm. This shows the benefits of stretching the fibers twice, first in an alcohol water bath and second in a water bath. These results led to the creation of our two-stretch mechanical system.

Double-Stretch Bath System

For the second bath in the double-stretch system, two different mixtures were used: dDI water and a 90:10 water:IPA mixture. In initial tests, the 100% dDI water bath produced fibers that had tensile strengths 30% higher and strain values 85% higher than the 90:10 water:IPA bath. Therefore, for all experiments a 100% dDI water was used as the second stretch-bath.

Researchers have used varying ratios of isopropanol and water to treat and improve synthetic fibers.^{22,23,44,53} To determine the optimal ratio of isopropanol and water, we spun fibers with isopropanol:water ratios of 90:10, 80:20, 70:30, 60:40, and 50:50 in the first bath. Maximum tensile strength was the desired property. Results can be seen in Figure S1. A

ratio of 70:30 IPA:water resulted in fibers with the highest tensile strength, and all further experiments utilized this IPA:water ratio.

Additionally, other researchers have treated spider silk films with methanol, and seen improved properties.^{29–31} As such, a mixture of methanol and water at a ratio of 80:20 MeOH:water was used in the first bath of the three godet double stretch system. For both the IPA:water bath fibers and the MeOH:water bath fibers the same stretch ratios were collected. In the first bath the fibers were stretched to 1.5× their original length and in the second bath fibers were stretched to 1.5X their original length, now labeled as 1.5× 1.5×. For the second stretch ratio, the stretch in the second bath was increased to 2X, labeled as 1.5× 2×. The 1.5× 2× fibers end up 3× the original as-spun length, making them directly comparable to the single bath system. The results of these experiments can be seen in Table 3. For both MeOH:water and IPA:water, as the stretch was increased the tensile strength of the fibers increased but strain decreased. The 1.5× 1.5× fiber for the IPA:water bath had significant strain, 1.253 ± 0.28 mm/mm, which was close to the value of the single stretch in 50:50 IPA:water. However, the tensile strength of the 1.5× 1.5× was 115.8 ± 9.7 MPa, nearly twice that of the 50:50 IPA:water stretched fibers. The 1.5× 1.5× MeOH:water fibers had higher tensile strength than any of the single stretch fibers, at 164.1 ± 23.9 MPa. The strain was similar to that of the water dipped fibers, measuring 0.662 ± 0.13 mm/mm.

Figure 6 shows the stress vs strain curves for the single stretch in comparison to the 1.5× 2× fibers spun with the double stretch system. Fibers processed with the IPA:water bath had a lower tensile strength, measuring 128.8 ± 20.8 MPa, than the single stretched fibers. However, the strain of the IPA:water fibers measured 0.877 ± 0.12 mm/mm, which is over three times the value of the strain of the single stretch fibers. As a result, the toughness was approximately three times that of the single stretch fibers, with a value 95.9 ± 18.2 MJ/m³. Methanol:water stretched fibers had a higher tensile strength, 221.7 ± 11.0 MPa, than either the IPA:water or single stretch fibers. The strain was 0.560 ± 0.07 mm/mm, not as high as the IPA:water fibers but nearly double that of the single stretch fibers. Figure 7 shows a comparison of the IPA:water and MeOH:water fibers. Some general trends can be observed in these fibers. As tensile strength is increased, by using MeOH:water as a stretch-bath, there is a loss of strain. However, the toughness of both is nearly identical, with MeOH:water fibers at 102.46 MJ/m³ and the IPA:water fibers at 95.9 MJ/m³.

Table 4 shows the standard deviations as a percentage of the average for commercial synthetic fibers, the synthetic spider silk fibers produced by other researchers and the double stretch samples presented in this study. The standard deviation for other synthetic spider silks is high; sometimes the standard deviation was larger than the average, most likely due to the nature of how the fibers were processed. The standard deviation of max stress for KEVLAR and para-aramids were 13.73 and 12.98% respectively. The standard deviation of toughness was 24.89 and 27.17%, respectively. These results were higher than was expected for commercial products. Nylon and polyester had smaller deviations. For stress, the standard deviation was 3.80 and 3.31% for nylon and polyester and for toughness the values were 8.15 and 15.99%. The MeOH:water double stretched fibers had standard deviations similar to those of nylon and polyester, with toughness and stress deviations of 13.26 and 4.97%, respectively. The IPA:water stretched fibers had standard deviations closer to those

of KEVLAR and para-aramid, with toughness and stress reporting deviations of 19.01 and 16.17%, respectively. These results show that the double-stretch system is capable of producing fibers with consistent mechanical properties, similar to industrially produced manmade synthetics.

XRD data (Figure 8) on the as-spun and double-stretched fibers helps to explain the differences between the IPA:water bath and the MeOH:water bath. The as-spun fibers show a single ring much like those seen in as-spun synthetic spider silks previously published. The IPA:water double stretched fibers show a much thinner ring with a center approximately equal to the as-spun along with more reflections, both isotropic and anisotropic. However, there is very little of the (120) reflections and no (200) reflections seen in the IPA:water samples. This indicates that there is some recruitment and orientation of β -sheet structures within the IPA:water stretched samples. The MeOH:water double stretched fibers not only show anisotropic and isotropic reflections typical of those seen in natural silks,⁵⁴ the (120) and (200) reflections can be seen. The total XRD data indicates some increase of β -sheet structures with the IPA:water stretched fibers, but much better recruitment and orientation in the MeOH:water stretched fibers. These results correlate with the mechanical properties of the fibers from both stretch baths and help to explain on a molecular level the reason for the differences between IPA:water stretched fibers and MeOH:water stretched fibers.

CONCLUSION

Previously, synthetic spider silk fibers were spun and then hand-drawn. That method, although useful for studying the feasibility of creating synthetic silk fibers, is ultimately highly variable and not industrially viable. This study describes the evolution of a process for spinning synthetic spider silk fibers which makes use of a mechanical system with the ability to create fibers with consistent mechanical properties. Changing the conditions under which the fibers are processed alters the mechanical properties of the fibers. In addition to changing the protein sequence of synthetic spider silks, changing the resultant fiber through processing adds an alternate means by which synthetic fibers can be tailored to meet the specific needs of defined commercial applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Diagram of the DACA SpinLine that has been modified for single-bath stretching of fibers.

Figure 2.
Diagram of the DACA SpinLine that has been modified for multiple stretching of fibers.

Figure 3.
Microscope images of (a) an as-spun fiber and (b) a stretched fiber.

Figure 4. Stress vs strain curves for a comparison of the stretch ratios used on the single stretch mechanical system.

Figure 5. Mechanical testing data from synthetic fibers stretched made with the mechanical single-bath system. The column charts represent maximum stress, maximum strain, diameter, and toughness of the fibers. Error bars represent the standard deviation of each group, with n 10.

Figure 6. Comparison of single mechanical stretch and the double-stretch system. All samples have been stretched to 3× their original length.

Figure 7. Mechanical testing data from synthetic fibers stretched using a dual-stretch system. The column charts represent maximum stress, maximum strain, Young's modulus, and toughness of the fibers. Error bars represent the standard deviation of each group, with $n = 10$.

Figure 8.

(a) XRD pattern for as-spun synthetic spider silk. (b) XRD pattern for IPA:water stretched synthetic spider silk. (c) XRD pattern for MeOH:water treated synthetic spider silk.

Table 1

Mechanical Properties of Synthetic Spider Silk Fibers As-Spun and with a Post-Spin Draw (PSD) Applied by Hand

author and citation	state	post-spin method	tensile strength (MPa)	strain (%)	toughness (MJ/m ³)
An et. al ⁴²	as spun		35.6	3.1	0.87
	PSD	3×, 75% IPA	132.5	22.8	23.73
An et. al ²⁶	as spun		16.2	3.1	0.24
	PSD	3×, 75% IPA	28.1	26.6	6.09
Teulé et.al ²²	as spun		28.4	1.7	0.3
	PSD	3×, 75% IPA	101.7	18.7	18.8
Albertson et. al ²³	as spun		13.6	1.2	0.1
	PSD	4×, 85% IPA	53.2	306.7	109.73
Heidebrecht et. al ⁴⁵	as spun		13	6	2
	PSD	6×, 75% IPA	370	110	189
Adrianos et. al ⁴⁴	as spun		26.3	0.7	0.06
	PSD	3×, 80% IPA	150.6	84.5	89.05

Table 2

Mechanical Properties of Fibers Processed on the Single-Stretch Mechanical System in Different Stretch Bath Compositions^a

	stress (MPa)	strain (mm/mm)	toughness (MJ/m³)
as-spun	32.57	0.011	0.18
std. deviation	7.44	0.003	0.07
80:20 IPA:H ₂ O bath	143.16	0.258	34.63
std. deviation	13.46	0.106	0.43
50:50 IPA:H ₂ O bath	59.34	1.430	61.64
std. deviation	11.80	0.226	9.82
2 M ammonium sulfate bath	145.45	0.553	66.00
std. deviation	15.99	0.129	13.58

^a80:20 IPA:H₂O and 2 M ammonium sulfate samples were stretched to three times their original length whereas the 50:50 IPA:H₂O bath samples were stretched to 2.5 times their original length. 2 M ammonium sulfate samples were also rinsed in water after being spun to remove any salt from the surface of the fibers.

Table 3

Mechanical Properties of Synthetic Spider Silk Fibers Processed with a Double-Bath System

	diameter (μm)	stress (MPa)	strain (mm/mm)	toughness (MJ/m ³)
as-spun				
no stretch	60.62	32.57	0.011	0.18
std. deviation	1.86	7.44	0.003	0.07
IPA:water stretch				
1.5 $\times$ 1.5 $\times$	35.06	115.77	1.253	123.44
std. deviation	1.44	9.66	0.283	29.97
1.5 $\times$ 2 $\times$	32.88	128.80	0.877	95.89
std. deviation	2.65	20.83	0.116	18.23
MeOH:water stretch				
1.5 $\times$ 1.5 $\times$	35.57	164.12	0.662	92.34
std. deviation	2.87	23.87	0.125	25.35
1.5 $\times$ 2 $\times$	28.98	221.72	0.560	102.46
std. deviation	1.12	11.01	0.066	13.59

Table 4

Table Showing Standard Deviation As a Percent of the Average of Synthetic Spider Silk Fibers from Various Studies, Commercial Synthetic Fibers, and Fibers Created in This Study^a

material	standard deviation		
	toughness	stress	strain
KEVLAR	24.89	13.73	12.59
para-aramid	27.17	12.98	14.98
nylon	8.15	3.80	5.01
polyester	15.99	3.31	10.19
synthetic spider silk by Teulé et. al	110.11	32.25	95.72
synthetic spider silk by Bo et. al	77.79	37.12	83.67
synthetic spider silk by Heidebrecht et. al	21.81	17.14	18.18
MeOH:water 1.5× 2× fibers	13.26	4.97	11.78
IPA:water 1.5× 2× fibers	19.01	16.17	13.22

^aThe commercial fibers and fibers from this study used $n = 10$ for calculations of standard deviation and the other values are as reported.