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Polymer 45 (2004) 3973–3977
polymer
www.elsevier.com/locate/polymer

Structure and morphology of electrospun silk nanofibers

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Received 21 May 2003; received in revised form 14 October 2003; accepted 14 October 2003

Dedicated to Professor Richard Boyd on the occasion of his 74th birthday and retirement, May 2003

Abstract

Nanoscale fibers of natural silks of *Bombyx mori* and *Nephila clavipes* were produced from solutions in hexafluoro-2-propanol. The electrospun fibers were observed by optical, scanning electron, and transmission electron microscopy. These nanofibers showed optical retardation, appeared to have a circular cross-section, and were thermally stable under nitrogen to 280 °C (*N. clavipes*) and to 245 °C (*B. mori*). The diameter of the fibers ranged from approximately 6.5–200 nm making them orders of magnitudes smaller than the natural silks spun by most silkworms and spiders. The smallest fiber diameters correspond to 200 molecules in the cross section of the *N. clavipes* fibers and 150 in *B. mori*. Electron diffraction patterns of annealed electrospun fibers of *B. mori* and *N. clavipes* exhibit diffraction peaks demonstrating orientational and crystalline order comparable to that of naturally spun silks.

Keywords: *N. clavipes*; *B. mori*; Electrospinning

1. Introduction

The useful physical properties of natural silk fibers [1] and the ability to produce these proteins using biotechnology [2], have provided the impetus for the efforts in both the biosynthesis and the spinning of these proteins. A great many organisms produce silk. Silkworms and spiders are two main ones. Silkworm silk, of the domesticated *Bombyx mori*, is well known and has been of interest for at least 5000 years due to its importance as a textile fiber [3,4]. In comparison, the study of spider silks is relatively new. Orb weaving spiders are sophisticated users of silk. Some possess up to seven different sets of glands which produce as many different fibers, all designed for specialized applications [5]. The most prominent set is the major ampulate which produces the protein for dragline silk. One of the most carefully studied spider silk fibers is the dragline of the orb weaver *Nephila clavipes*. This is partially due to the spider's large size and the associated relatively large quantities of silk it can produce. Dragline fibers produced by the orb weaver exhibit a highly desirable combination of strength, toughness and compressibility, not evident in synthetic high-performance fibers [6]. Some synthetic polymers such as Kevlar® have a higher strength than *N. clavipes* dragline, but their toughness is significantly lower [1].

There were three objectives of this work. The first was to completely dissolve the *B. mori* cocoon and *N. clavipes* dragline silk fibers while maintaining the polymeric character of the silks. Second, to develop a system for re-spinning the resulting silk solutions by the process of electrospinning. Third, to produce natural silk fibers with diameters that are several orders of magnitude smaller than the original fibers, suitable for direct observation and analysis using transmission electron microscopy (TEM) and electron diffraction as well as scanning electron microscopy (SEM).

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doi:10.1016/j.polymer.2003.10.102

2. Experimental

2.1. Materials

2.1.1. Silk fibers

Dragline silk was obtained from adult female *N. clavipes* spiders, generously supplied by Mark Stowe, (University of Florida, Gainsville, FL). The spiders were housed individually and fed a diet of live crickets. The method of silk collection and the apparatus used were similar to one described previously [7] and not too different from one described before 1807 [8]. The draw rate was 1.1 cm/s, which is not too different from the natural spinning rate [9] and the filaments were collected onto Teflon sheets. Degummed cocoon silk of the *B. mori* (Shinakebono) was kindly provided by the laboratories of Drs Jun and Yoshiko Magoshi, (Tsukuba, Japan).

2.1.2. Silk solubilization

The *N. clavipes* dragline silk samples were placed in sterile glass bottles. Hexafluoro-2-propanol (Aldrich 99% $(CF_3)_2CHOH$) was added to a final concentration in the range of 0.23–1.2% (weight percent). The bottle was sealed to prevent solvent evaporation. The silk dissolved within 20 min at room temperature. The solution was used immediately for spinning. It remained stable and was also used successfully a few months later for spinning.

The *B. mori* silk was cut into lengths of a few millimeters and placed in a sterile glass bottle. Hexafluoro-2-propanol was added to a final concentration of 0.74% (weight percent). No visible dissolution was observed at room temperature. The bottle was sealed and set aside. The fibers eventually dissolved after a period of 5 months at room temperature.

2.2. Electrospinning

In the electrospinning process, a high electric potential is applied to a droplet of a polymer solution at the tip of a syringe needle. When the electric force from the applied field becomes larger than the surface tension of the polymer droplet, a charged jet of the polymer solution is formed and ejected in the direction of the applied field. The jet dries as the solvent is evaporated in the air. The dried fibers are collected on a receiving conducting mesh. In our experiment, the silk solution was contained in a Hamilton syringe (25 μl) driven by a compact infusion pump (Model No. 975, Harvard Apparatus Co. Inc., Denver, MA.). The collecting mesh was placed at a distance of about 15 cm from the syringe tip, and perpendicular to the syringe axis. A voltage of 24–30 kV was applied to the mesh by a high voltage power supply, while the tip of the syringe was grounded. The process was carried out at room temperature and the spinning rate was controlled by adjusting the flow of the silk solution and the electrical field. The voltage was reduced just enough to eliminate the corona discharge which occurs at high field strengths [10,11].

2.3. Characterization

2.3.1. TEM

TEM grids were mounted on the metal receiving mesh, where dry silk fibers were collected. The grids were then placed on a glass slide and annealed in a Mettler FP82HT hot stage under flowing nitrogen gas. The fibers were coated with a very thin layer of evaporated carbon, before observation in the transmission electron microscope (JEOL JEM-1200EXII), to avoid electrical charging. Low dose techniques were used in the TEM to minimize radiation damage to the fibers.

2.3.2. SEM

SEM sample holders were lined with silicon wafers and mounted on the metal receiving mesh where the dry silk fibers were collected. The samples were coated and observed using a (Hitachi S-900) Low Voltage High Resolution Scanning Electron Microscope (LVHRSEM). Diameters were measured along the visible length of randomly selected fibers in many images. The diameters of the occasional large 'beads' along the fibers were not included.

2.3.3. Wide angle X-ray diffraction (WAXD)

WAXD experiments were conducted with a Rigaku 12 kW rotating-anode generator (Cu K_α) coupled with a Bruker (Siemens) Hi-Star area detector running a general area detector diffraction software (GADDS) version 3.2. The peak positions and widths were calibrated with silicon crystals of known crystal size using the (111) plane. Background scattering was recorded and subtracted from the WAXD patterns.

To prepare the sample of the as-spun dragline fibers, a taut fiber bundle was created by wrapping strands of the dragline fibers around two pins mounted on top of a cardboard frame. When the desired thickness was obtained, the ends of the bundle were glued securely to the top and bottom of the cardboard frame and allowed to dry. The cardboard frame was then mounted onto an X-ray sample holder. The bundle of degummed cocoon fibers was prepared for the WAXD experiment in the same way as the *N. clavipes* as-spun fibers.

3. Results and discussion

Electrospinning produced a non-woven sheet of randomly arranged silk fibers with nanometer scale diameters. The electrospun fibers showed some birefringence when observed between crossed polars. Some are uniform in diameter and some are not. The fibers of *N. clavipes* appear to have a rounded cross section and a smooth surface when

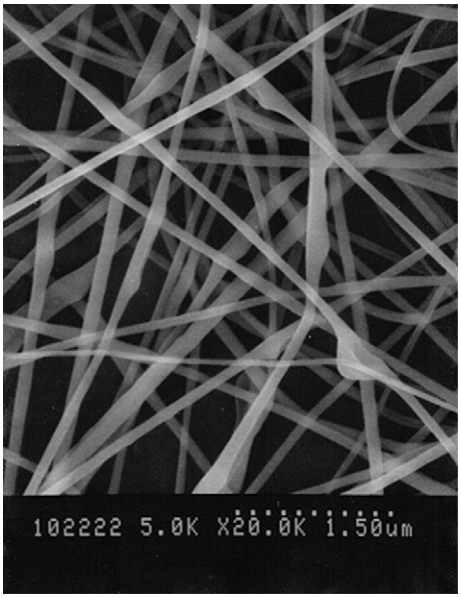

Fig. 1. LVHRSEM micrograph of fibers electrospun from *N. clavipes* silk solution.

observed at high magnifications by LVHRSEM (Fig. 1). Their diameters exhibited a size range of approximately 8–200 nm with 100 nm being the most frequently occurring (Fig. 2), making them orders of magnitude smaller than most of the naturally produced fibers (~2–5 μm), but of the same order as nanofibers that have been reported as sub-fibers that exist in natural silk [12]. They are within the range of reported sizes for fibers electrospun from other polymers [13]. The fibers produced with the silk of *B. mori* are similar in appearance (Fig. 3), but a little smaller. Fig. 4 shows the smallest observed fiber which at 6.5 nm is in the range of the smaller ones reported for electrospining [13]. The overall size range is approximately 6.5–100 nm with

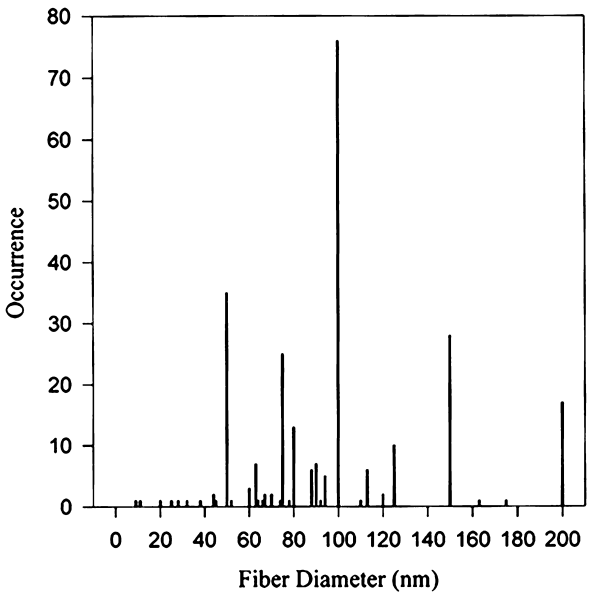

Fig. 2. Size distribution of fibers electrospun from *N. clavipes* silk solution.

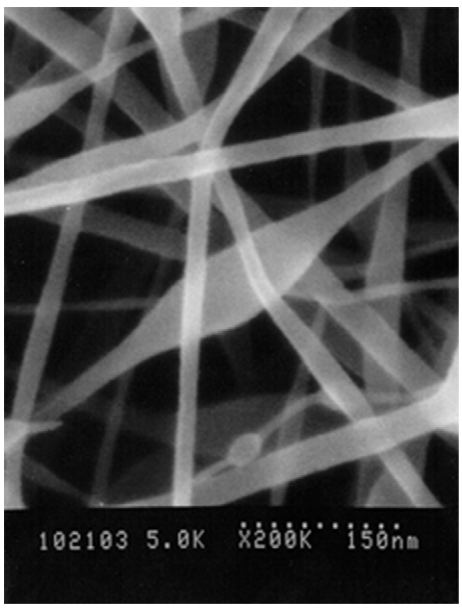

Fig. 3. LVHRSEM micrograph of fibers electrospun from *B. mori* silk solution.

25 nm being the most frequent (Fig. 5). For this reason, they were observed at a higher magnification. They too are smaller than the natural fibers and of the same order as the nanofibers reported as sub-fibers [14]. Further, they also are in the range of sizes of fibers electrospun from other polymers [13]. The smallest observed diameters correspond to approximately 200 molecules in the cross section of the fibers of *N. clavipes* and 150 in *B. mori*. These numbers were obtained by dividing the fiber cross sectional areas by the unit cell areas of 1 and 0.88 nm^2 for the two silks, respectively, [15] and multiplying by the four molecules in the unit cells.

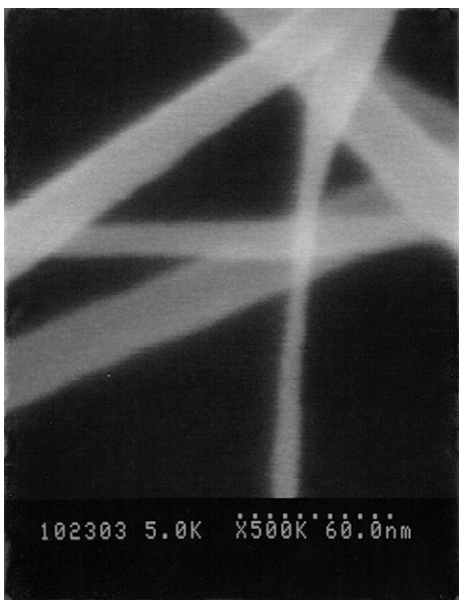

Fig. 4. LVHRSEM micrograph of fibers electrospun from *B. mori* silk solution.

Table 1
Electron and WAX diffraction from *N. clavipes* dragline silk

hkl	Electron-electrospun		WAXD-As-spun natural	
	Intensity	*d*-Spacing (nm)	Intensity	*d*-Spacing (nm)
120, 210	Strong	0.451	Strong	0.438
200	Medium	0.582	Strong	0.537
121, 211, 021	Weak	0.372	Medium	0.366

Electron diffraction performed on unannealed fibers of both silks did not result in a crystalline diffraction pattern, but simply a diffuse halo at approximately 4.4 Å. Therefore, the molecular arrangement of the as-spun fibers was somewhat disordered and kinematically determined. The fibers of *N. clavipes* were annealed under flowing nitrogen gas at temperatures ranging from 50–280 °C. After annealing in a small window of temperatures from 210–230 °C, oriented crystalline diffraction was observed for single fibers. At annealing temperatures above 280C °, the fibers began to show degradation. They became yellow/-orange in color and, as seen by optical microscopy, curled and broken. *B. mori* electrospun fibers were annealed under flowing nitrogen at temperatures ranging from 150–300 °. For a small, but somewhat broader window of annealing temperatures from 205–240 °C, oriented crystalline diffraction was observed. Above 245 °C there was no silk crystalline diffraction and the fibers became yellow/orange. Unlike the *N. clavipes* electrospun fibers, they showed no sign of curling and breaking. At 300 °C a new crystalline pattern appeared that was robust to the electron beam, but was not from silk.

The *N. clavipes* electrospun fibers produced two equatorial diffraction spots corresponding to spacings of 0.58 nm, and 0.45 nm as well as a non-equatorial one corresponding to 0.37 nm (Table 1). The diffraction patterns indicate preferred orientation of the molecular axis of the crystals along the fiber axis. WAXD data were obtained for bundles of the as-spun natural fibers (Table 1). (Electron diffraction patterns cannot be obtained from the natural single fibers. Because the large size of the fibers prevents penetration by the electron beam.) The fitted unit cell parameters for both the electron diffraction and WAXD results agreed within two standard deviations or less with one another and with the values for category 3b silk which includes *N. clavipes* (Table 2) [15]. When diffraction was

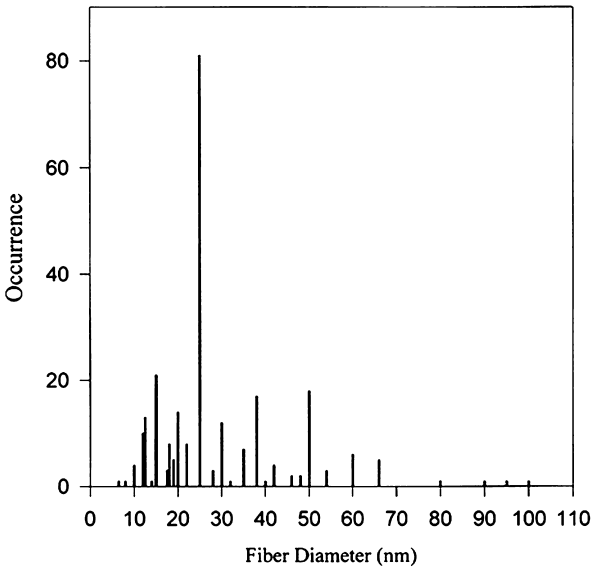

Fig. 5. Size distribution of fibers electrospun from *B. mori* silk solution.

Table 2
Fitted unit cell dimensions for *N. clavipes* dragline silk

Warwicker [15]	Parameter	Electron-electrospun		WAXD-As-spun natural	
		Dimension (nm)	Standard deviation (nm)	Dimension (nm)	Standard deviation (nm)
1.06	*a*	1.095	0.049	1.039	0.036
0.944	*b*	0.98	0.054	0.955	0.042
0.695	*c*	0.61	0.06	0.61	0.05

Table 3
Electron and WAX diffraction from *B. mori* cocoon silk

hkl	Electron-electrospun		*hkl*	WAXD-As-spun natural	
	Intensity	*d*-Spacing (nm)		Intensity	*d*-Spacing (nm)
120, 210	Strongest	0.427	120, 210, 200	Strong	0.458
200	Strong	0.467	–	–	–
121, 211, 021	Strong	0.38	121, 211, 021	Medium	0.372
–	–	–	002	Medium	0.366
003	Weak	0.212	–	–	–
006	Weak	0.117	–	–	–
–	–	–	102	Weak	0.326

Table 4
Fitted unit cell dimensions for *B. mori* silk

Warwicker [15]	Parameter	Electron-electrospun		WAXD-As-spun natural	
		Dimension (nm)	Standard deviation (nm)	Dimension (nm)	Standard deviation (nm)
0.93	*a*	0.95	0.02	0.96	0.04
0.944	*b*	0.97	0.03	0.99	0.05
0.695	*c*	0.67	0.03	0.70	0.03

performed on the electrospun fibers at long camera length (240 cm), a low angle equatorial streak was observed.

The *B. mori* electrospun fibers produced two equatorial diffraction spots as well as a non-equatorial one and two meridianal ones (Table 3). The electron diffraction patterns indicate preferred orientation of crystals along the fiber axis. The fitted results agree within one standard deviations or less with one another and with the values for category 1 silk which includes *B. mori* (Table 4).

4. Conclusions

The silks of the *N. clavipes* spider and the *B. mori* silkworm were dissolved, while preserving their polymeric nature. Nano-scale fibers were spun from these solutions by the electrospinning technique. The as-spun fibers exhibited only diffuse electron diffraction. After annealing, these fibers show crystallographic order similar to that observed in the original natural fibers. The fibers of *N. clavipes* are thermally stable to about 280 °C and the fibers of *B. mori* to about 245 °C. The importance of this technique is that it provides a way of producing silk fibers that are orders of magnitude smaller than whole fibers produced by nature (*B. mori* $\sim 10-20\ \mu m$ and *N. clavipes* $\sim 2-5\ \mu m$). Conventional methods such as wet-spinning, dry-jet wet spinning, or dry-spinning techniques produce fibers with larger diameters [16]. The electrospun nanofibers are of a similar diameter as the sub-fibers of silk that have been observed [12,14]. Due to their small diameter, the entire nanofiber can be observed by electron diffraction without the need for microtoming or other destructive sample preparation procedures. The availability of nanoscale silk fibers introduces a new set of possible uses of these amazing fibers at a scale not explored before. New uses include small diameter fibers for filtration of sub-micron particles in vivo, nanocomposite reinforcing fibers for nanotechnology, sutures, and other bio-medical applications.

Acknowledgements

The authors thank Mr Joe Williams of the Wright Patterson Air Force Base for his generous help and expertise with the LVHRSEM. They also thank the reviewers for helpful comments and suggestions. Portions of this work have been presented to a meeting of the American Chemical Society [10,11].

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