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Polymer 47 (2006) 5630–5632
www.elsevier.com/locate/polymer

Electrospun *Bombyx mori* gland silk

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Received 1 June 2005; accepted 28 June 2005
Available online 6 June 2006

Dedicated to Prof. David Bassett on the occasion of his retirement

Abstract

Solutions of *Bombyx mori* gland silk can be electrospun with the addition of some polyethylene oxide (PEO). Green fluorescent protein (GFP) can also be incorporated and electrospun without apparent phase separation from the silk. The dimensions of the fibers with and without the GFP are qualitatively similar. The results indicate the possibility of making fibers with uniform non-linear optical properties.

Keywords: GFP; Electrospun gland silk; Non-linear optical properties

1. Introduction

Since the electrospinning of silk was first reported [1], there has been an increasing interest in such work. However, all the efforts have used reconstituted silks from biologically spun fibers. In this short note, we report the use of *Bombyx mori* silk dissolved in water directly from the gland and spun. Some PEO is added to the solution to facilitate spinning.

We have previously shown that GFP, which exhibits non-linear optical properties can be incorporated into cast silk films [2]. However, the GFP and silk phases separated somewhat thereby yielding non-uniform results in optical transmission measurements. Therefore, experiments to determine whether GFP molecules could be incorporated into electrospun gland silk fibers were done. It was hypothesized that the relatively large size of the GFP molecule [3] compared to the size of the fibers [1] might limit the possible separation. Further, the large surface area to volume ratio of the fibers was expected to speed the evaporation of the water, thereby quickly reducing the GFP mobility and hence reducing the opportunity for phase separation.

2. Experimental

The eggs of *B. mori* were obtained from NIAS, CREST, Japan. The worms were hatched in a laboratory at about 24 °C and at 36–42% RH. They were fed fresh mulberry leaves and reared according to USDA regulations. After the fourth moulting, but before cocoon spinning, the silk gland was removed and rinsed in distilled water. Thereafter, the membrane was removed and the contents were slowly dissolved in deionized water at 5 °C. The GFP was made using an encoding Vector produced by Clontech. It was purified [4] and the 2.03 mg/ml solution was stored at −20 °C. Solutions of silk, water, PEO and GFP were made with the compositions shown in Table 1.

The solutions were electrospun onto glass slides or aluminum foil with a 1 mm diameter spinneret. The spinneret to the aluminum foil or glass sample collection plate distance was 40 cm and the spinning voltage was 40 kV [5]. Both sparse and dense layers of fibers were produced. They were observed with bright field optical microscopy using Olympus BX-60 and BX-51 microscopes. Ultraviolet illumination with wavelength ranges of 360–490 nm and 460—490 nm, respectively, was used in the reflection mode (epifluorescence). SEM images

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0032-3861/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.polymer.2005.06.130

Table 1
Composition of the solutions

Sample no.	Silk (g)/PEO (g)	GFP (mg)/(Silk+PEO) (g)
1	25.017	0
2	0.642	8.855

were made with either a JEOL Model USM 5310 or an FEI Model Quanta 200. The fiber dimensions were measured using Image J software.

3. Results and discussion

Electrospun fibers prepared from the silk and PEO solution (sample number 1) and observed under visible light are shown in Fig. 1. The beads that sometimes form on the fibers can be eliminated by variation of the spinning parameters [6]. Fig. 2 is a higher magnification SEM image. The fibers are in the range of 80–270 nm as can be seen in the histogram in Fig. 3. The average dimension of the fibers is 158±46 nm.

Fig. 4a is an image observed under visible light of a sparse layer of electrospun fibers made by adding the GFP solution to the silk and PEO solution (sample number 2). Fig. 4b shows an image of the same area when viewed under UV light (epifluorescence image). It clearly shows that GFP has been incorporated relatively uniformly in the fibers without indication of phase separation. Fig. 5a shows an optical image and Fig. 5b shows an epifluorescence image of a thicker and denser layer of fibers made with sample number 2. Comparison of specific fibers in Fig. 5a and b leads to the same conclusion as was reached for the sparser film in Fig. 4a and b. The brighter spots in Fig. 5b could be eliminated by variation of the spinning parameters [6]. None of the fibers exhibited evidence of phase separation of the GFP and silk. Fig. 6 shows a higher magnification SEM image of the fibers made from sample number 2. The fibers made from the solutions with GFP (Fig. 7) are qualitatively similar to the ones made without GFP. The average dimension of the fibers is 170±47 nm.

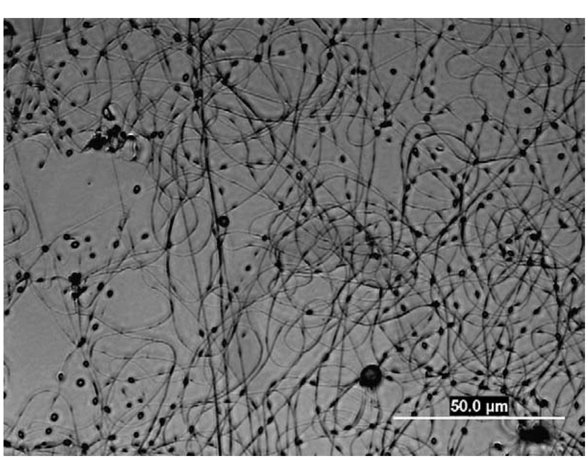

Fig. 1. An optical image of electrospun fibers made from a solution of gland silk and PEO (sample number 1).

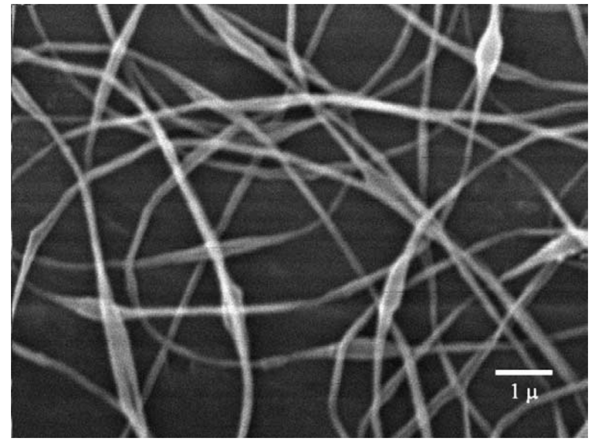

Fig. 2. An SEM image of electrospun fibers made from a solution of gland silk and PEO (sample number 1).

4. Conclusions

Solutions of *B. mori* gland silk can be electrospun with the addition of some PEO to facilitate the spinning. The results show that GFP can be incorporated relatively uniformly in the fibers without phase separation. This indicates the possibility of making fibers with uniform non-linear optical properties.

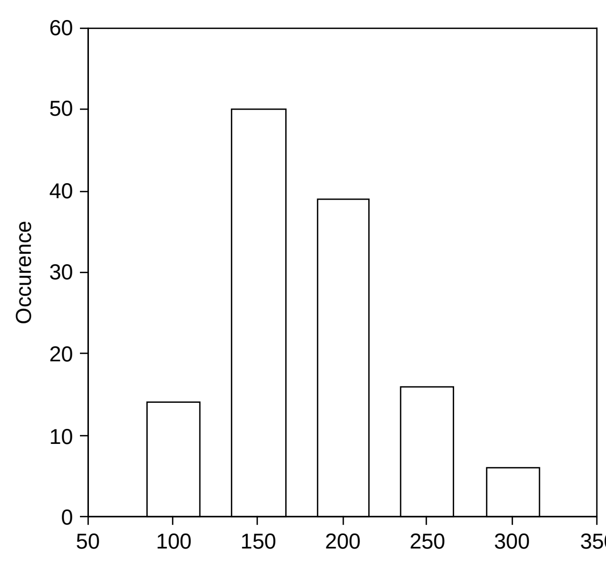

Fig. 3. Histogram of fiber dimensions from sample number 1.

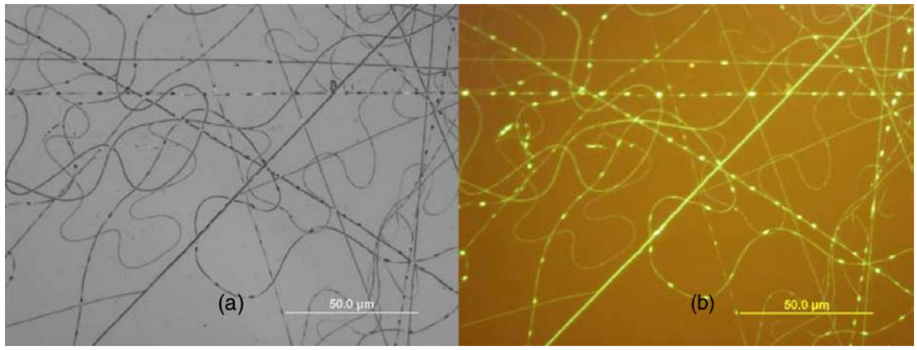

Fig. 4. Optical images of a sparse layer of sample number 2 under visible light (a) and UV light (epifluorescence). Images (a) and (b) are from the same area.

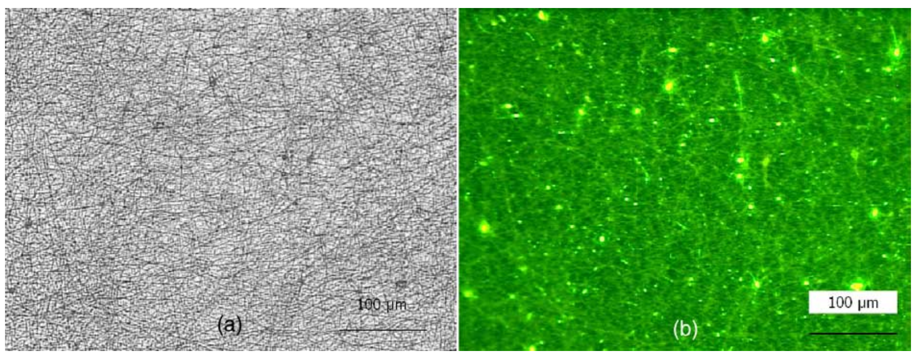

Fig. 5. Optical images of a thicker and denser layer of fibers made from sample number 2 under visible light (a) and UV light (epifluorescence). Images (a) and (b) are from the same area.

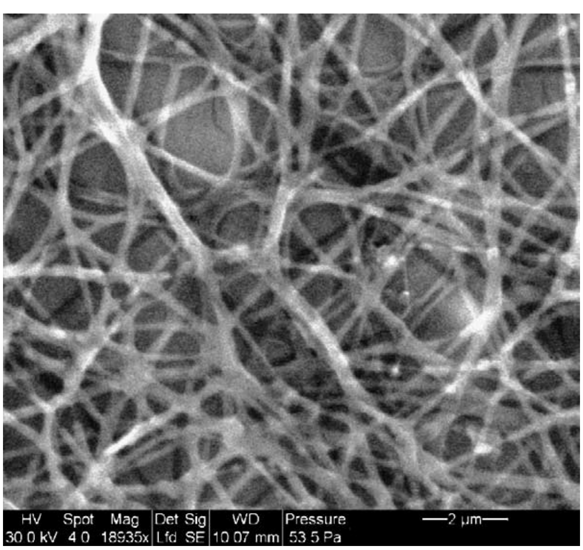

Fig. 6. An SEM image of electrospun fibers made from sample number 2.

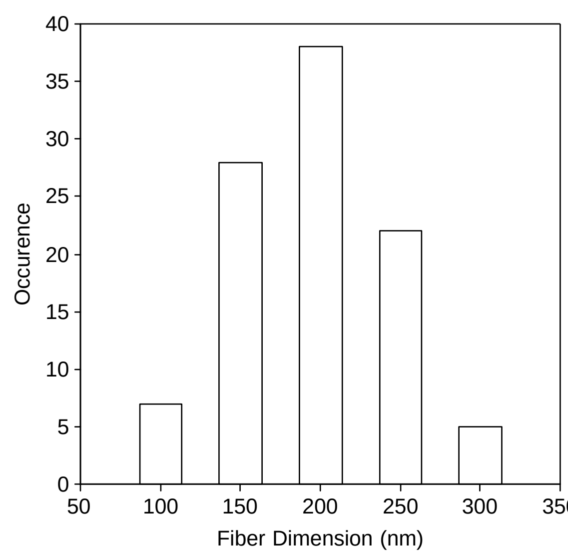

Fig. 7. Histogram of fiber dimensions from sample number 2.

Acknowledgements

The authors acknowledge the support of AFOSR award no. F49620-03-1-0169. They also thank Y. Magoshi and J. Magoshi of NIAS, Tsukuba, Japan, and Prof. M. Becker, Fukui University, Japan.

References

[1] Zarkoob S, Reneker DH, Eby RK, Hudson SD, Ertly D, Adams WW. Polym Preprint 1998;39(2):244.
[2] Putthanarat S, Eby RK, Naik RR, Juhl SB, Walker MA, Peterman E, et al. Polymer 2004;45:8451.
[3] Shimomura O, Johnson FH, Saiga Y. J Cell Comp Physiol 1962;59:223.
[4] Yakhnin AV, Vinokurov LM, Surin AK, Alakslov YB. Protein Expr Purif 1998;14(3):382.
[5] Yarin AL, Koombhongse S, Reneker DH. J Appl Phys 2001;89(5):3018.
[6] Fong H, Chun I, Reneker I. Polymer 1999;40:4585.