Available online at www.ijtnbm.ir

The Iranian Journal of Textile Nano-bio Modificatio

IJTNBM 2 (2022) 39–44

Effects of changes in some electrospinning parameters on fibroin nanofibers morphology

Amirasad Pourabadeh [a*], Shaghayegh Sadrzadeh

Department of Research and Development, ABRISHAM MAHAN DELIJAN Co., Tehran, Iran

Received 19 July 2022; accepted 19 August 2022

Abstract

Silk fibroin (SF) is a natural fibrous protein that has been widely studied for application in the biomedical field as a matrix for tissue engineering. Pure fibroin was extracted from silk cocoon by degumming method using aqueous Na_2CO_3 solution followed by solubilizing in $CaCl_2$-C_2H_5OH-H_2O aqueous solution and frozen in liquid nitrogen, then lyophilized in freeze-dryer. SF fibers with diameters down to the nanometer range are formed by subjecting a fluid jet to a high electric field. The electrospinning of the SF sponge was performed with formic acid, as a spinning solvent, at 7% (w/w) fibroin concentration. In this regard, electrospinning parameters including voltage, flow rate, and distance were used as variable parameters, and the effect of changes in these parameters was investigated. The morphology of SF nanofibers was characterized by SEM. As the flow-rate increased, the available polymer volume was high which increased the nanofiber diameter. Also the lower the solution flow-rate, the smaller the diameter of the resultant electrospun nanofibers and bead defects. With an increase in the distance between the capillary and the collector the nanofiber diameter initially decreased to a minimum and then increased.

Keywords: Silk Fibroin, Electrospinning, Nanofiber, Cell culture, Tissue engineering.

[*]* Corresponding author. Tel:.
E-mail address:

1. Introduction

Natural silk from the silkworm has been used as a biomedical suture material for centuries [1]. Silk proteins are produced by various species of silkworms, spiders, scorpions, and bees. Silk fibers are built by two proteins, namely, fibroin and sericin. Sericin is the gummy substance surrounding fibroin. For biomedical applications only silk fibroin is useful, sericin due to its immunogenicity needs to be removed [2]. It has been reported that the biological features of fibroin make it an excellent choice for use in cell attachment, proliferation, and tissue regeneration [3, 8]. Fibroin has been widely explored in many biomedical applications due to its impressive biocompatibility, biodegradability, and minimal inflammatory reactions [4, 5]. Electrospinning is a unique method capable of producing nanoscale fibers from both synthetic as well as natural polymers for biomedical applications [6, 7].

In the present study, silk was dissolved in CaCl2-C2H5OH-H2O to obtain a silk fibroin solution. The fibroin solution was frozen in liquid nitrogen, and then lyophilized in a freeze-dryer. The electrospinning of the SF sponge was performed with formic acid as a spinning solvent. To characterize the changes in some electrospinning parameters of fibroin nanofibers including voltage, flow rate, and distance, the morphology of nanofibers prepared by electrospinning was studied. The morphology of the SF nanofibers was evaluated by scanning electron microscopy (SEM).

2. Materials and Methods

2.1. Preparation of Fibroin

Silk fibers are obtained from Bombyx Mori silkworm cocoons and were washed twice with distilled water. In order to remove sericin coating from silk fibers, 20 g of silk in 2.5 L of a 0.01 M solution of Na2SO3 in distilled water were boiled for 40 min, then the degummed fibers were washed thoroughly. Fibers were allowed to dry in a flow cabin. Silk fibers were solubilized in CaCl2-C2H5OH-H2O=1:2:8 (molar ratio), for 6h at 75°C. The solution was taken in a stirrer/hot plate at 50 RPM and was strained to remove the not dissolved fibers. CaCl2 was removed in a stirred cell for 8 h (Stirred Cell Model 8010, Amicon, Merck, Germany) using a membrane disc of ultra-cell regenerated cellulose of 100 kDa (Merck, Germany). The SF solution was instantaneously frozen in liquid nitrogen (-196 °C) and then lyophilized for 24 hours in a Liobras® freeze-dryer (model L101, Brazil) to obtain the SF sponge.

2.2. Electrospinning

The SF electrospinning solution was prepared by dissolving the SF sponge in 98% formic acid (Merck) and stirred at room temperature for 4 h to obtain complete dissolution. In the electrospinning process, the SF solutions were placed into the 2.5 mL syringe with a stainless needle connected to a high voltage power suppy.

2.3. Characterizing Technique

The morphology of the SF nanofibers scaffold was observed, using SEM (S-5700, Hitachi, Tokyo, Japan). The intact samples were coated with gold for SEM witnessing. In the SEM photos, the fiber diameters were determined using Image J software and the results were given as the average diameter ± standard deviation. In this regard, electrospinning parameters including voltage, flow rate, and distance were used as variable parameters, and the effect of changes in these parameters was investigated.

3. **Results and Discussion**

3.1. Effect of Changes in the Applied Voltage

We observed that the difference between the applied voltage that would cause a polymer drop to become unstable and that which would cause it to become conical in shape is very small. Any further increase in voltage beyond a critical value leads to the ejection of a polymer jet from the apex of the cone. As clearly shown from SEM images in Fig 1, this critical value of applied voltage was 25 kV. A voltage that is weaker or stronger than this critical value will result in beaded morphologies or even inhibit polymer jet initiation. With an increase in the applied voltage, the nanofiber diameter increases. The increase in nanofiber diameter is attributed to a higher polymer mass of jet stretching in correlation to increased charge repulsion within the jet and a strong external electric field as a consequence of an increase in the applied voltage.

Table 1. Characteristic of samples with different voltage in electrospinning of fibroin

NO. / **Parameter**	**1**	**2**	**3**	**4**	**5**
Voltage (kV)	21	23	25	27	**29**
Flow rate (ml/h)	1	1	1	1	**1**
Distance (cm)	10	10	10	10	**10**

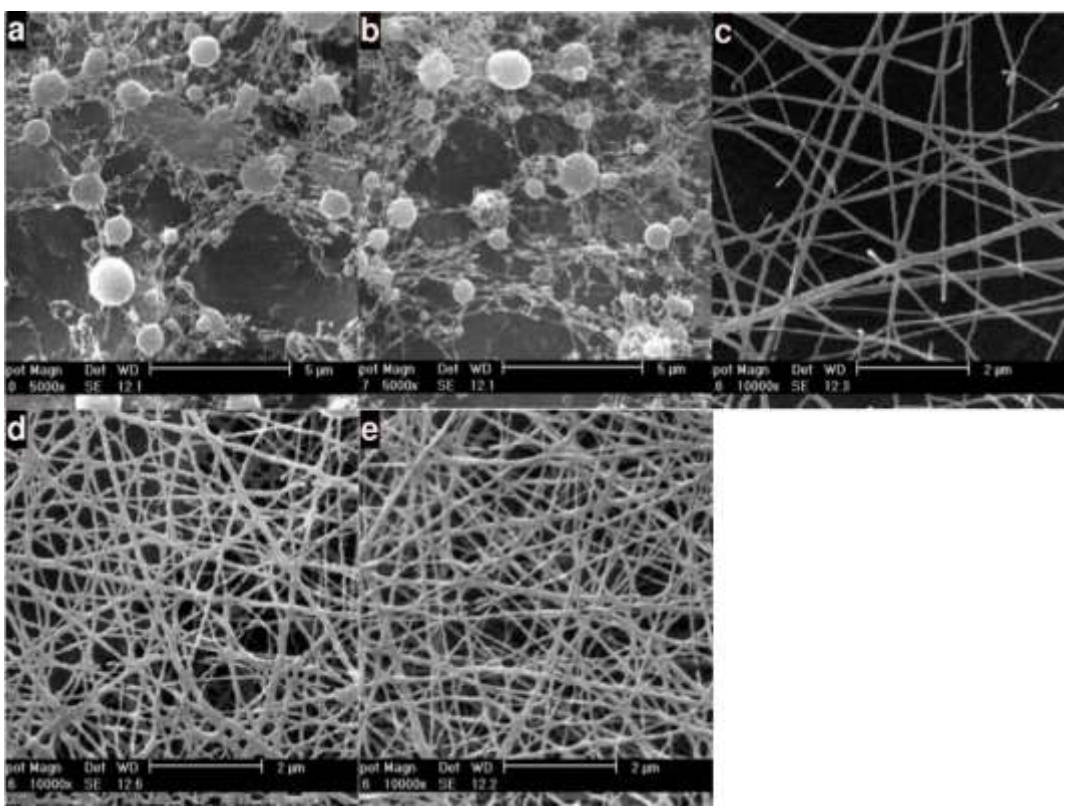

Figure 1. Morphologies of SF Nanofibers at Different voltage: a. 21; b. 23; c. 25; d. 27; e. 29 (kV).

3.2. Effect of changes in the Solution Flow-Rate

The flow-rate of the polymer solution through a capillary influence the nanofiber diameter, porosity, and geometry of the electrospun nanofibers. To maintain the Taylor Cone shape at the capillary tip and avoid bead defects, a minimum flow-rate of the polymer is required to replace the solution that is lost when the nanofiber jet is ejected. As shown in Fig 2, the minimum flow-rate was 1.2 ml/h.

As the flow-rate increased, the available polymer volume was high which increased the nanofiber diameter. When the flow-rate was too high, the nanofibers were unable to dry completely before reaching the collector and higher bead defects were therefore observed. Flattened ribbon-like nanofiber morphology also results from incomplete drying of nanofibers due to a high flow-rate.

It was shown that the lower the solution flow-rate, the smaller the diameter of the resultant electrospun nanofibers and bead defects.

Table 2. Characteristic of samples with different flow rate in electrospinning of fibroin

NO.	**1**	**2**	**3**	**4**	**5**
Parameter					
Voltage (kV)	27	27	27	27	**27**
Flow rate (ml/h)	1	1.1	1.2	1.3	**1.4**
Distance (cm)	10	10	10	10	**10**

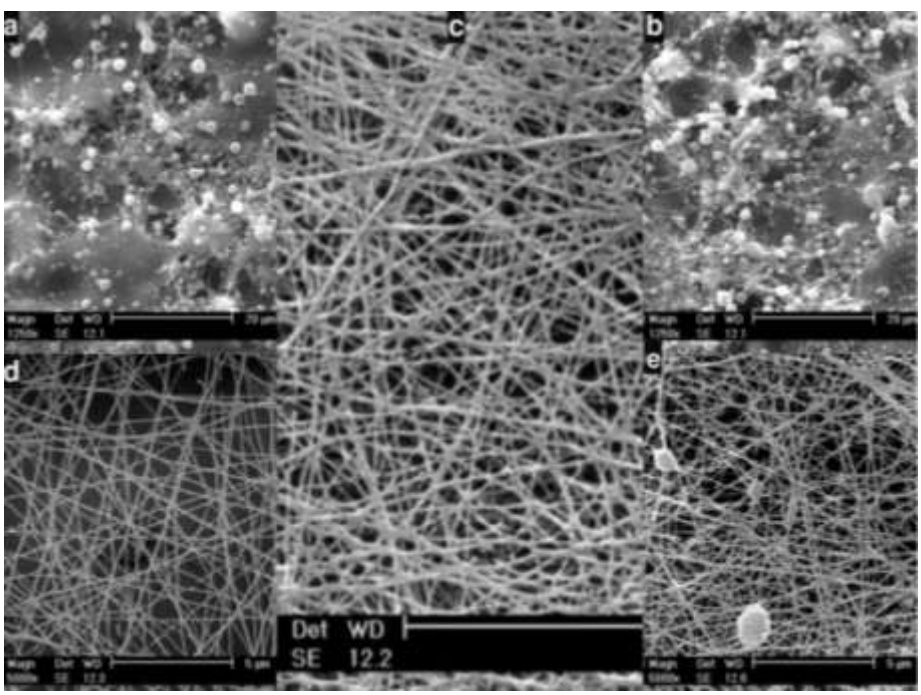

Figure 2. Morphologies of SF Nanofibers at Different Flow-Rate: a. 1.0; b. 1.1; c. 1.2; d. 1.3; e. 1.4 (ml/h).

3.3. Effect of changes in the Collector Distance

The capillary to collector distance influences the size and morphology of the nanofibers formed. However, its effect is relatively less profound compared to the other processing variables discussed. An optimum distance between the capillary and collector is desirable for nanofiber formation and on either side of this range bead formation or electro spraying instead of electrospinning may be observed.

Fig.3 shows the microstructure of SF electrospun at different Collector Distances. As shown in Figure 3, with an increase in the distance between the capillary and the collector the diameter of electrospun nanofibers decreases. At smaller distances, the solvent does not have sufficient time to evaporate completely resulting in nanofibers with flattened structures due to inadequate drying. Also, we observed initiation of bead formation as the distance very decreased. These results clearly indicate a decrease in nanofiber diameter with an increase in the distance up to 130 mm, beyond which the fiber jet became too small and unstable.

Table 3. Characteristic of samples with different distance in electrospinning of fibroin

NO.	**1**	**2**	**3**	**4**	**5**
Parameter					
Voltage (kV)	27	27	27	27	**27**
Flow rate (ml/h)	1.2	1.2	1.2	1.2	**1.2**
Distance (cm)	9	10	11	12	**13**

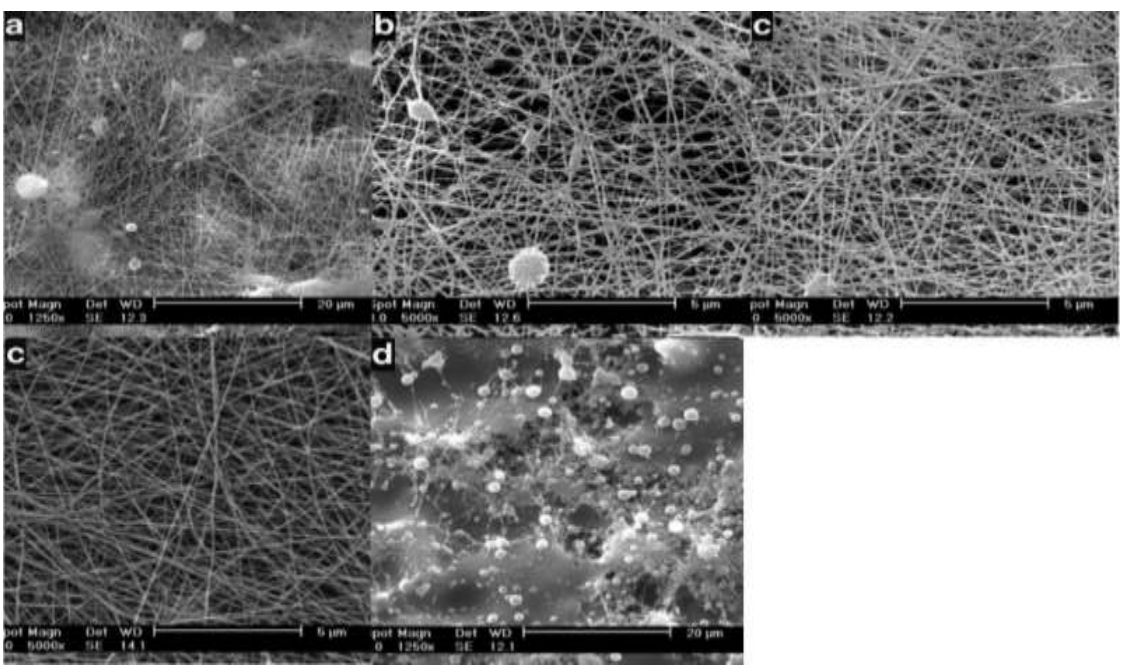

Figure 3. Morphologies of SF Nanofibers at Different Collector Distance: a. 9 cm; b. 10 cm; c. 11 cm; d. 12 cm; e. 13 cm.

4. Conclusions

In this paper, the effects of changes in Voltage, Rate, and Distance on the morphology of Silk Fibroin Nanofibers were investigated. As observed by SEM results to investigate the effect of an increase in the applied voltage, the nanofiber diameter increases. The increase in nanofiber diameter is attributed to a higher polymer mass of jet stretching in correlation to increased charge repulsion within the jet and a strong external electric field as a consequence of an increase in the applied voltage.

As the flow-rate increased, the available polymer volume was high which increased the nanofiber diameter. This was attributed to the larger droplet at the end of the capillary, due to the very higher flow-rate, resulting in the solution having a faster trajectory and resulting in incomplete drying and the formation of bead defects. Also, the lower the solution flow-rate, the smaller the diameter of the resultant electrospun nanofibers and bead defects.

With an increase in the distance between the capillary and the collector the nanofiber diameter initially decreased to a minimum and then increased. At smaller distances, the solvent does not have sufficient time to evaporate completely resulting in nanofibers with the formation of beads.

References

1. S.N. Fan, Y.P. Zhang, H.L. Shao, et al. Electrospun regenerated silk fibroin mats with enhanced mechanical properties [J]. International Journal of Biological Macromolecules, 2013, 56: 83-88.
2. B. Kundu, N.E. Kurland, S. Bano, et al. Silk proteins for biomedical applications: bioengineering perspectives [J]. Progress in Polymer Science, 2014, 39: 251-267.
3. C. Vepari, D.L. Kaplan. Silk as a biomaterial [J]. Progress in Polymer Science, 2007, 32(8-9): 991-1007.
4. M.F. Zhu, K. Wang, J.J. Mei, et al. Fabrication of highly interconnected porous silk fibroin scaffolds for potential use as vascular grafts [J]. Acta Biomaterialia, 2014, 10(5): 2014-2023.
5. C.S. Ki, S.Y. Park, H.J. Kim, et al. Development of 3-D nanofibrous fibroin scaffold with high porosity by electrospinning: implications for bone regeneration [J]. Biotechnol Letter, 2008, 30: 405-410.
6. J. Ayutsede, M. Gandhi, S. Sukigara, et al. Regeneration of Bombyx mori silk by electrospinning. Part 3: characterization of electrospun nonwoven mat [J]. Polymer, 2005, 46(5): 1625-1634.
7. Z. Liu, F. Zhang, J.F. Ming, et al. Preparation of Electrospun Silk Fibroin Nanofibers from Solutions Containing Native Silk Fibers [J]. Applied Polymer, 2014, 41236: 1-7.
8. P. Katta, M. Alessandro, R.D. Ramsier, et al. Continuous electrospinning of aligned polymer nanofibers onto a wire drum collector [J]. Nano Letters, 2014, 4(11): 2215-2218.