EFFECT OF NEEDLE DIAMETER ON DIAMETER OF ELECTROPSUN SILK FIBROIN NANOFIBERS

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Abstract: Electrospinning is a process that creates nanofibers through an electrically charged jet of polymer solution or polymer melt. The value of the technology lies in the smallest fiber diameters that can be fabricated because nanofibers offer more performance advantages in many fields as their diameters decrease. Many parameters, which are classified broadly into solution parameters, process parameters, and ambient parameters play significant roles in determining the morphology and diameter of electrospun nanofibers. In order to be able to have a control on fiber diameter, it is important to investigate the parameters that influence it. In this study, an attempt is made to investigate the effect of needle diameter on the resulting electrospun SF average nanofiber diameter by using four different needle gauges. The resulting nanofibers were analyzed by scanning electron microscopy (SEM) and statistically significant decrease in nanofiber diameter was observed in response to the decrease in needle diameter.

1. Introduction

Silks are fibrous proteins with remarkable mechanical properties produced in continuous fiber form by spiders and silkworms. Silk fibroin (SF) fiber, popularly known in the textile industry for its luster and mechanical properties, is produced by cultured silkworms [1]. It has been used as a surgical suture material for several centuries due to its good mechanical and biological properties including biocompatibility and low inflammatory reaction [2]. It has played an important role not only in political, cultural, and economic history (Silk Road, national costumes), but also in the history of technology (textile machines, Jacquard) and in science (protein chemistry, genetics, and genetic engineering). Despite the triumphal advance of synthetic fibers, silk maintains its place in the raw material market, in the textile and clothing industries, and in the retail trade because of its unique properties [3]. Besides its traditional use in textiles, the range of applications of silkworm silk is expanding mainly in the field of biomaterials due to their intrinsic properties as biocompatibility, biodegradability and mechanical superiority [3,4]. Several different material morphologies such as gels, sponges, films, and nanowebs produced through regeneration of silk fibroin (SF) have been applied in a wide variety of biomedical applications such as cell or tissue scaffolds, drug delivery carriers, wound dressing etc. [1,2,5]. Especially the availability of silk nanofibers with high surface area to volume ratio and highly porous three dimensional structure through a simple process of electrospinning introduces a new set of potential uses.

Electrospinning is a process that creates nanofibers through an electrically charged jet of polymer solution or polymer melt. Polymer solution or the melt that has to be spun is forced through a syringe pump to form a drop of the polymer at the tip of the syringe needle. High voltage potential is applied to the tip of the needle, thereby inducing free charges into the polymer solution. These charged ions move in response to the applied electric field towards the electrode of opposite polarity, thereby transferring tensile forces to the polymer solution. At the tip of the needle, the polymer drop takes a cone like projection in the presence of an electric field. And, when the applied potential reaches a critical value required to overcome the surface tension of the liquid, a jet of liquid is ejected from the cone tip. After the initiation from the cone, the discharged polymer solution jet undergoes an instability and elongation process, which allows the jet to become very long and thin and is field directed towards the oppositively charged collector [6,7]. As the jet travels through the atmosphere, the solvent evaporates, leaving behind a dry fiber on the collecting device [8]. Electrospinning process is governed by many parameters, classified broadly into solution parameters, process parameters, and ambient parameters. Solution parameters include concentration, molecular weight, viscosity, surface tension, conductivity, polymer solubility; process parameters include applied voltage, tip to collector distance, type of collector, diameter of needle, flow rate, and ambient parameters include the humidity and temperature of the surroundings each of which plays a significant role in determining the morphology and diameter of electrospun nanofibers [6,9]. Since the value of the technology lies in the smallest fiber diameters that can be fabricated, it is important to investigate the parameters that influence the fiber diameter in order to be able to have a control on fiber diameter.

Silk was first electrospun and patented by Zarkoob *et al.* in 2000 [10]. Afterwards, there have been some simultaneous efforts to characterize the structure and morphology of nanofibers as a function of solution and process parameters, and to investigate the use of silk fibroin in different applications [11-22]. However, no study has been performed investigating the effect of needle diameter on the diameter of electrospun silk fibroin nanofibers. In this study, the effect of needle diameter on the resulting electrospun silk fibroin average nanofiber diameter has been evaluated for four different needle gauges.

2. Experimental

2.1. Materials and Equipment

'A' quality, dried *B. mori* silkworm cocoons were cut open and the pupas were removed before the process. Then the cocoons were cut into small pieces. Na_2CO_3, $CaCl_2.2H_2O$, CH_3CH_2OH, 98% formic acid were supplied from domestic suppliers. Slide-A-Lyzer G2 Dialysis Cassettes with MWCO of 3.5K and sample volume capacity of 30 mL were used for the fibroin purification. They were supplied from Thermo Fisher Scientific Inc. Freeze dryer of Christ ALPHA 1-4 LD Plus type was used for the removal of water from the dialyzed aqueous fibroin solution.

2.2. Preparation of regenerated silk fibroin

B. mori silk fibroin was prepared by boiling 25 g of cocoons in 500 mL aqueous solution of Na_2CO_3 (2 g/L) for 40 min, rinsing thoroughly with distilled water to extract the glue-like sericin proteins and air-drying. Then 5 grams of silk fibroin was dissolved in 50 mL of $CaCl_2/H_2O/CH_3CH_2OH$ (mole ratio 1:8:2) solution at 80ºC in 40 minutes. The fibroin solution was filtered and dialyzed against distilled water for 3 days, using Slide-A-Lyzer dialysis cassettes to remove $CaCl_2$ and CH_3CH_2OH. After filtrating, the clear fibroin aqueous solution was obtained and it was freeze-dried to obtain regenerated SF sponges. The freeze-drying was carried out at three stages as freezing at -25 ºC for 24 h, primary drying at -40 ºC for 24 h and secondary drying at -60 ºC for 24 h.

2.3. Preparation of the electrospinning solutions

SF solution with concentration of 18% by weight was prepared by dissolving the regenerated SF sponges in 98% formic acid for 3 hours.

2.4. Electrospinning

The electrospinning setup was parallel positioned and consisted of a high voltage power supply which could supply positive voltage from 0 to 30 kV, a syringe pump, and a rectangular (10×10cm) aluminum foil collecting plate. The syringe was filled with 5 mL of fibroin solution and high voltage of 15 kV was applied to the tip of the needle. Constant volume flow rate of 0,018 mL/h was maintained using a syringe pump. The fibers were collected on the aluminum foil which was set at 10 cm distance from the tip of the needle. 22, 20, 19, 18G needles, which had diameters of 0.70, 0.90, 1.06, 1.25 mm. respectively, were used for the investigation of needle diameter on silk fibroin nanofiber (Table 1).

Table 1: *List of samples produced for the investigation of needle diameter effect on fiber diameter, together with the electrospinning parameters*

Sample No.	Concentration (wt.%)	Needle gauge (G) - Diameter (mm)	Voltage (kV)	Flow rate (mL/h)	Tip-to-collector distance (cm)
S1	18%	22G – 0.70mm	15	0.018	10
S2	18%	20G – 0.90mm	15	0.018	10
S3	18%	19G – 1.06mm	15	0.018	10
S4	18%	18G – 1.25mm	15	0.018	10

2.5. Characterization

The fiber diameters were examined by field emission scanning electron microscope (Supra 50 VP FESEM) after the nanowebs had been coated with gold. The fiber diameters were measured from multiple SEM images, and 100 fibers were analyzed per experiment. The diameters were measured using Analyzing Digital Images Software.

2.6. Statistical Analysis

The fiber diameter distributions were assumed as normal to carry out statistical analysis on data. SPSS 19.0 (**S**tatistical **P**ackage for the **S**ocial **S**ciences) program was used for statistical analysis and One-way ANOVA ($\alpha = 0.05$) was applied in order to approve whether the differences between mean diameters were of real importance. $P<0.05$ was considered statistically significant.

3. Results and Discussion

1,000, 5,000 and 50,000 times magnified SEM micrographs of samples S1, S2, S3 and S4 are presented in Figure 1a–d, respectively.

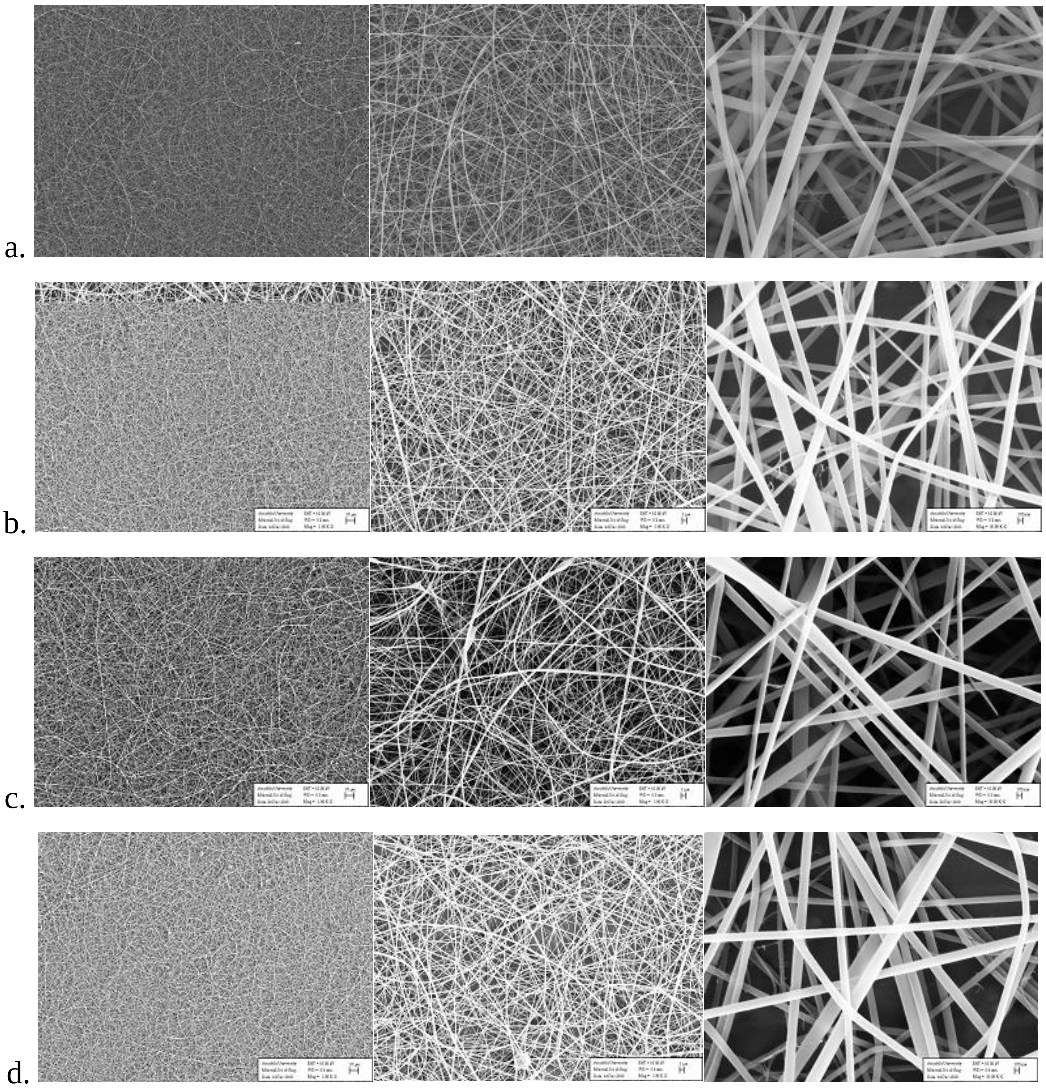

Figure 1 *SEM images of samples a.S1; b.S2; c.S3; d.S4*

Uniform fibers were obtained from the solution with a concentration of 18wt%. From the SEM images of nanowebs produced from the solution with a concentration of 18wt%, it is clear that the change in needle diameter in the mentioned range did not lead to bead formation.

The resulting silk fibroin nanowebs were analyzed to calculate the average nanofiber diameter and statistical descriptives in order to investigate the correlation between needle diameter and average fiber diameter. The mean fiber diameters and descriptives can be seen in Table 2.

Table 2: *Descriptives for nanowebs produced from 18wt% SF solution*

Sample No.	Needle Gauge (Needle Diameter)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min.	Max.
						Lower Bound	Upper Bound		
S1	22G (0.70mm)	100	149.778	50.78154	5.07815	139.7018	159.854	50.00	325.00
S2	20G (0.90mm)	100	155.336	48.09470	4.80947	145.7930	164.879	56.30	325.00
S3	19G (1.06mm)	100	162.438	57.29938	5.72994	151.0681	173.807	56.25	331.25
S4	18G (1.25mm)	100	171.464	58.26897	5.82690	159.9022	183.026	56.30	325.00
Total		400	159.754	54.19268	2.70963	154.4269	165.081	50.00	331.25

The mean fiber diameters were calculated as 149.8, 155.3, 162.4 and 171.5 nm for the samples S1, S2, S3, and S4, respectively which suggested a correlation between needle diameter and fiber diameter.

The variance analysis was carried out by applying Oneway Anova Method to decide whether the differences were of real importance (Table 3).

Table 3: *Oneway Anova table for the mean fiber diameters of the samples S1, S2, S3, and S4*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26336.457	3	8778.819	3.035	0.029
Within Groups	1145465.264	396	2892.589		
Total	1171801.721	399			

From variance analysis, it is concluded that the differences between the mean diameters were of real significance, since significance value was 0.029.

Multiple comparisons (Table 4) showed that the needle diameter change from 0.70 to 0.90 mm, 0.70 to 1.06 mm, 0.90 to 1.06 mm and 1.06 to 1.25 mm resulted in increases which were not of real significance ($\text{Sig.}_{0.70-0.90} = 0.465 > 0.05$, $\text{Sig.}_{0.70-1.06} = 0.097 > 0.05$, $\text{Sig.}_{0.90-1.06} = 0.351 > 0.05$, $\text{Sig.}_{1.06-1.25} = 0.236 > 0.05$) whereas the needle diameter change from 0.70 to 1.25 mm and 0.90 to 1.25 mm resulted in significant increases in fiber diameter ($\text{Sig.}_{0.70-1.25} = 0.005 < 0.05$, $\text{Sig.}_{0.90-1.25} = 0.035 < 0.05$).

The increase in fiber diameter as a result of increase in needle diameter is attributed to the increased size of the droplet at the tip of needle. When the size of the droplet at the tip of the needle increases, the surface tension of the droplet decreases. For the same voltage supplied, a smaller columbic force is required to cause jet initiation. As a result, the acceleration of the jet increases and this allows less time for the solution to be stretched and elongated before it is collected [6] which results in increased fiber diameters.

Table 4: *Multiple comparisons of the mean fiber diameters of nanowebs produced with 22, 20, 19, 18G needles from 18wt% SF solution*

(I) Needle Dia. (mm)	(J) Needle Dia. (mm)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0.70	0.90	-5.55800	7.60604	0.465	-20.5113	9.3953
	1.06	-12.65950	7.60604	0.097	-27.6128	2.2938
	1.25	-21.68600*	7.60604	0.005	-36.6393	-6.7327
0.90	0.70	5.55800	7.60604	0.465	-9.3953	20.5113
	1.06	-7.10150	7.60604	0.351	-22.0548	7.8518
	1.25	-16.12800*	7.60604	0.035	-31.0813	-1.1747
1.06	0.70	12.65950	7.60604	0.097	-2.2938	27.6128
	0.90	7.10150	7.60604	0.351	-7.8518	22.0548
	1.25	-9.02650	7.60604	0.236	-23.9798	5.9268
1.25	0.70	21.68600*	7.60604	0.005	6.7327	36.6393
	0.90	16.12800*	7.60604	0.035	1.1747	31.0813
	1.06	9.02650	7.60604	0.236	-5.9268	23.9798

*. The mean difference is significant at the 0.05 level.

4. Conclusion

Silk nanofibers were obtained utilizing different gauge needles and fiber diameters were analyzed from the SEM images taken from the samples. The results indicated that there was a correlation between the needle diameter used and the average nanofiber diameter obtained. The average fiber diameters increased with increase in needle diameter. Utilization of needles with the minimum possible diameters which permits the problem free process may contribute to producing finer nanofibers.

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