Fabrication and Mechanical Characterization of Thai Silk Fibroin Nanofibrous Scaffolds

[Sathit Banthuek, Somchai Pattana]

Abstract—**Silk fibroin from Thai Bombyx mori (Nangnoi Srisaket-I) cocoons was interested to use as biomaterial for tissue engineering scaffolds. In this paper, Thai silk fibroin based nanofibrous scaffolds were fabricated by electrospinning technique. The effects of electrospinning parameters, such as silk fibroin/formic acid solutions concentrations and applied voltage, on the morphology and mechanical properties of Thai silk fibroin based nanofibrous scaffold were investigated. Thai Silk fibroin (SF) sponges were dissolved in 98% formic acid with concentration of 20, 30, and 40 % (w/v) and spun into fibers at applied voltage of 15, 20, and 25 kV, respectively. The morphological and mechanical properties of SF-nanofibers were examined by field emission scanning electron microscopy (FESEM) and AFM indentation method. Electrospinning technique could produce nanofibers in the range of diameter from 100–300 nm. The concentration of silk fibroin solution was considered as the most important parameter for regeneration of Thai silk fibroin nanofibers. The average diameter of the electrospun SF-nanofibers was found to increase with the increase in the solution concentration. Furthermore, the fiber diameter has also been considered as the main factor to influence the mechanical properties of silk fibroin nanofibrous scaffold. These characteristic could provide the utility data of Thai silk fibroin nanofiners for tissue engineering applications.**

  Keywords—**silk fibroin, nanofibrous scaffold, electrospinning, mechanical properties, tissue engineering**

I.   Introduction

Nanotechnology, especially nanofibers, has become attracted interest for tissue engineering application [1]. The large surface area to volume ratio is the unique properties of nanofibers, which consents cellular migration and proliferation in tissue engineering scaffolds [2]. The scaffold can be fabricated by several methods including solvent casting, particle leaching, phase separation, emulsion freeze drying, 3D-printing technique and many others [3]. However, the simplicity of electrospinning provides an advantage for nanofibrous scaffold construction [4].

Electrospinning is the most efficient method to fabricate continuous nanofibers from both synthetic and natural polymers. This method can produce polymer fibers with diameters ranging from micrometer down to nanometers [5].

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Due to the most of extracellular matrix (ECM) of natural tissues is composed of a cross-linked network of collagen fibrils with diameter in the range from 15 µm down to 5 nm, which human cell deposit in and organize around these fibrils [6]. Thus, electrospinning technique has been interest for artificial matrices fabrication in tissue engineering field due to its capability to produce nanofibrous scaffold mimicking fibrous structure of native ECM [7].

In general, the key component for tissue engineering is required biocompatible and biodegradable scaffolds with different characteristics [8]. Presently, polymeric biomaterials are used for scaffolds construction such as poly-lactic acid, poly-glycolic acid, and collagen. However, some of these scaffold materials have incomplete mechanical strength and regularly induce an inflammatory response [8, 9]. A protein based polymer, silk has been extensively used as high-quality textile fibers and biomedical suture material for centuries [10]. Silks are commonly define as fibrous proteins that produced by a variety of insects such as silkworms, spiders, and flies. Silk fibroin from Bombyx mori silkworm exhibits several useful properties that are suitable for scaffolding application including good biocompatibility, good biodegradability, excellent mechanical durability, and less inflammatory reaction [11-14]. Moreover, silk-based nanofibers also present high specific surface area to volume ratio, increased strength and enhanced thermal and electrical conductivity [15].

In the recent years, numerous reports were focused on the electrospinning of silk fibroin and the used of electrospun silk fibroin fiber mats as scaffold for tissue and cell culture [16]. Recently, silk fibroin from some Thai Bombyx mori silk, Nangnoi Srisaket-I, Nang-Lai, and Samrong, have been gradually applied for many fields [17-20]. In the electrospinning, the process parameters such as spinning distance, solution concentration and applied voltage has been examined as important factors to control the morphology and diameters of Thai silk fibroin nanofibers. However, the mechanical properties of electrospun nanofibers on different process parameters has not been investigated.

The objective of this study was to fabricate the nanofibrous scaffolds, and to investigate the effect of process parameters for silk electrospinning on the morphology and mechanical properties of electrospun Thai silk fibroin nanofibers. Thai Bombyx mori (Nangnoi Srisaket-I) silk was used as raw materials for the preparation of silk fibroin. The Thai silk fibroin sponges were dissolved in formic acid to prepare SF/formic acid solution. The solutions were spun into fibers via electrospinning technique at various conditions. The effects of the electrospinning parameters on the morphology and mechanical properties of obtained nanofibrous scaffolds were characterized and discussed.

II.   Materials and Methods

The local Thai silk cocoons, Bombyx mori (Nangnoi Srisaket-I) were obtained from Queen Sirikit Sericulture Center, Chiang Mai province, Thailand. All basic chemicals used in this study were purchased from Sigma-Aldrich.

A. *Preparation of Silk Fibroin Solution*

Thai B. mori cocoons were degummed in aqueous 0.02 M Na_2CO_3 at 100 °C for 30 min twice and rinsed with warm water in order to remove sericin. The degummed cocoons were dissolved in a ternary solvent solution of $CaCl_2/CH_3CH_2OH/H_2O$ (1: 2: 8 in molar ratio) at 70 °C for 6 h to obtain silk fibroin solution. The solution was poured into cellulose tubular membrane (Dialysis Tubing D9527, Sigma) and dialyzed in deionized water for 72 h at 4 °C. After dialysis process, the silk fibroin solution was lyophilized to obtain the silk fibroin (SF) sponges [21]. The SF sponge was dissolved in 98% formic acid for 3 h to prepare SF/formic acid solutions at 20, 30, 40 % (w/v) of concentrations, respectively.

B. *Electrospinning*

In the electrospinning process, the SF/formic acid solutions with concentration of 20, 30, and 40% (w/v) was placed in a 5 ml glass syringe (spinning angle 45°). The high electrical voltage (Gamma High voltage Research Inc. USA) at 15, 20, and 25 kV was applied to a droplet of SF solution at the tip (0.5 mm inner diameter) of a syringe needle. The tip-to-collected plate (covered with aluminum foil) was placed at a distance of 10 cm. The syringe tip and the collected plate were enclosed in a chamber to control the temperature of spinning process. Electrospun SF-nanofibers were immersed in 90% methanol for 60 min at room temperature and dried under vacuum for 24 h to yield the stabilized nanofibers.

C. *Morphology Observation and Fibers Measurement*

The morphology of the electrospun SF-nanofibers was visualized by field emission scanning electron microscope (FESEM) to observe the inner structure of scaffolds. The average fiber diameter of SF-nanofibers was determined from a measurement of 100 random fibers.

D. *Mechanical Properties of Electrospun Nanofibers*

The mechanical properties of the electrospun nonwoven mat were evaluated using the atomic force microscopy (AFM) indentation method. The electrospun mat was tested via AFM instrument (XE-70, Park Systems Corp., Suwon, Korea). AFM was controlled by a piezo translator, a maximum x-y scan range of 50 µm and a z-range of 12 µm. XE Data Acquisition Program was used for data analysis. A 130-µm-long Si_3N_4 cantilever (Park Systems Corp., Suwon, Korea) with a spring constant of 12 N/m was used for this experiment. In order to obtain the data, the relationship of the cantilever deflection (Δd) and indentation depth (Δz) were analyzed. Generally, the force curve was characterized by a flat area when the tip was approached on the sample and by a slope region where the cantilever deflection is conform to the z movement,

$$d = z \qquad (1)$$

In the case of a soft flat sample, due to the decrease in the deflection value was changed because of elastic indentation (δ),

$$d = z - \delta \qquad (2)$$

Hooke's law relates the deflection with the applied force through the force constant of the cantilever (k),

$$F = kd = k (z - \delta) \qquad (3)$$

From the relationship of the indentation, δ, with the loading force, F, of Hertz model and by using the Sneddon's modification model.

$$F= \qquad (4)$$

Where E is the Young's modulus, v is the Poisson ratio of the specimen. The Poisson ratio was assumed to be 0.5 for incompressible materials, and α is the opening angle of the AFM tip. By combining equations (3) and (4),

$$kd = \qquad (5)$$

With the rearrangement of equation (3) we have an expression for indentation (δ),

$$\delta = \qquad (6)$$

The indentation (δ) can be replaced by combining equations (1) and (6),

$$z = d + \qquad (7)$$

The data treatment was used as more general term,

$$z \quad z_o = (d \quad d_o) + \qquad (8)$$

d_o and z_o are the initial values of deflection and depth, respectively. Equation (8) was used to calculate the value of the Young's modulus, E, of scaffolds [22-24].

III. Results and Discussion

A. *Effect of the Concentration of SF/formic acid solution on the Morphology of Nanofibers*

The morphology of the SF-nanofibers was strongly affected by concentration of the solution. Figure 1(a) and 1(b) showed the FESEM images of SF-nanofibers prepared from SF/formic acid solution with concentration of 20 and 30% (w/v). The applied voltage was kept constant at 15 kV. For the SF/formic acid solution with concentration of 20% (w/v), SF-nanofibers were produced with average diameter of 185.44 ± 3.54 nm. When the solution concentration was 30% (w/v), the size of SF-nanofibers was increased with average diameter of 196.17 ± 3.54 nm. However, the viscosity of SF/formic acid solution became comparatively high, which made the spinning process unstable. Therefore, the resultant electrospun SF-nanofibers exhibited an irregular morphology. For the SF/formic acid solution with concentration of 40% (w/v) could not produce any SF-nanofibers because of the electrostatic
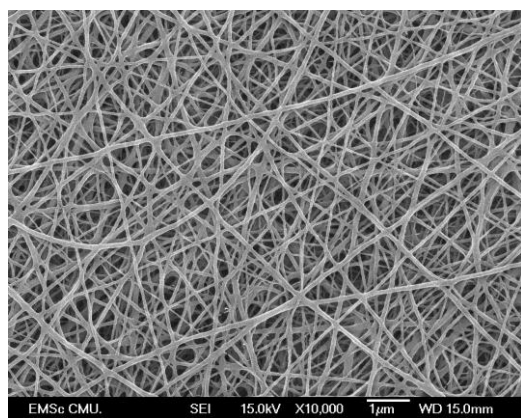
(a)
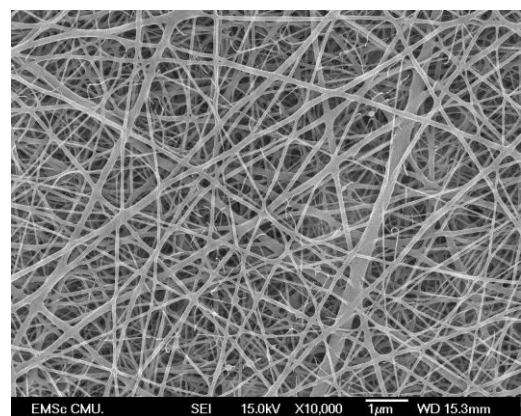
(b)

Figure 1. *FESEM images of electrospun SF-nanofibers, an applied voltage was 15 kV, prepared from SF/formic acid solution with concentration of (a) 20 % and (b) 30 % (w/v).*

TABLE I.   MORPHOLOGY OF ELECTROSPUN SF-NANOFIBERS

SF/formic acid concentration %(w/v)	Voltage (kV)	Diameter range (nm)	Average diameter (nm)
20	15	117 - 299	185.44 ± 3.54
	20	74 - 234	117.16 ± 2.88
	25	61 - 157	113.75 ± 2.82
30	15	108 - 272	196.17 ± 3.71
	20	71 - 181	107.16 ± 2.21
	25	63 - 252	108.44 ± 2.28

force could not overcome the surface tension of the SF/formic acid solution.

B. *Effect of the Applied Voltage on the Morphology of Nanofibers*

The effect of the applied voltage during electrospinning on SF-nanofibers was investigated. Figure 2 showed the FESEM images of SF-nanofibers prepared from SF/formic acid solution with concentration of 20, 30, and 40% (w/v) and applied voltage of 15, 20, and 25 kV, respectively. The morphology of electrospun SF-nanofibrous scaffolds was summarized in Table I. SF/formic acid solution with concentration of 40% (w/v) could not produce SF-nanofiber for any applied voltage. For the solution with concentration of 20% (w/v), SF-nanofibers were formed with smooth surface at applied voltage of 15 kV (Figure 2(a)). The intensity of the electrical field was increased by applying voltage of 20 kV and the spinning rate quickly increased, which made further elongate the jet into fibers. The morphology became decreased in size of fibers with average diameter of 117.16 ± 2.88 nm (Figure 2(b)). Because of the high elongation and spinning rate increased rapidly and when the intensity of the electric field was increased with applied voltage of 25 kV. The small diameter size of contracted SF-nanofibers with beads was exhibited (Figure 2(c)). Moreover, SF/formic acid solution in the jet did not have enough time to completely vaporize from the fiber surface before it reached at the collected plate and some of these fibers consequently became beaded fiber. Effect of the applied voltage on the morphology of the SF-nanofibers with concentration of 30% (w/v) provided similar results to 20% (w/v) solution concentration (Figure 2 (d), (e), and (f)). Average diameters of SF-nanofiber at various applied voltage of 15, 20, and 25 kV were 196.17 ± 3.71, 107.16 ± 2.21, and 108.44 ± 2.28 nm, respectively. For the same reason, the viscosity of the 30% SF/formic acid solution was comparatively high; it was not easy for solution in the jet to diffuse and vaporize from the fibers surface before they reached at the collected plate, and the SF-nanofibers became small diameter and beaded fiber again.

C. *Mechanical Properties*

The mechanical properties of the electrospun SF-nanofibers were determined by using atomic force microscope. The results of AFM indentation tests were shown in Figure 3. The modulus of the electrospun mats as calculated from

cantilever deflection (*d*) and vertical position (*z*) was presented in Table II.

The Young's modulus of electrospun SF-nanofibers prepared from SF/formic acid solution with concentration of 20% (w/v) and applied voltage at 15, 20, and 25 kV were 699.02 ± 27.33, 582.37 ± 18.17, and 561.35 ± 54.33 MPa while the SF/formic acid solution with concentration of 30% (w/v) were 739.08 ± 29.94, 557.26 ± 11.42, and 529.67 ± 13.49, respectively. The results showed that the fiber diameter

TABLE II.   MECHANICAL PROPERTIES OF ELECTROSPUN NANOFIBERS

SF/formic acid concentration % (w/v)	Voltage (kV)	Average diameter (nm)	Young's modulus (nm)
20	15	185.44 ± 3.54	699.02 ± 27.33
	20	117.16 ± 2.88	582.38 ± 18.17
	25	113.75 ± 2.82	561.35 ± 54.33
30	15	196.17 ± 3.71	739.08 ± 29.94
	20	107.16 ± 2.21	557.26 ± 11.42
	25	108.44 ± 2.28	529.67 ± 13.49

was an important factor to influence the mechanical properties of electrospun SF-nanofibers. Due to electrospun SF-nanofibers were formed as continuous fibers with smooth surface at applied voltage of 15 kV. This condition provided the good mechanical properties, both of 20 and 30% (w/v), when compared with others conditions. We found that decreasing size of the fiber diameter of electrospun SF-nanofibers was affected in decreasing of Young's modulus value as well.

IV.   Conclusions

Silk fibroin (SF) from cocoons of indigenous Thai Bombyx mori (Nangnoi Srisaket-I) was fabricated into nanofibers by electrospinning technique. The effect of process parameters including solution concentrations (i.e., 20–40% (w/v) in 98% formic acid) and applied voltages (i.e., 15, 20 and 25 kV) on morphology and mechanical properties of the electrospun SF nanofibrous scaffolds was investigated. The diameter size of the resulting electrospun SF-nanofibers in this study was in the range from 100–300 nm. When applied voltage was kept constant at 15 kV. At low solution concentrations, 20% (w/v), electrospinning of SF/formic acid solutions produced nanofibers with diameters in range of 185.44 ± 3.54 nm, while, at high solution concentrations, 30% (w/v), the diameter size of SF-nanofibers were increased with average diameter of 196.17 ± 3.54 nm. The morphology of the SF-nanofibers was found that the diameter of the electrospun nanofibers was evidently affected by concentration of the solution. For beaded fibers, an increase in the applied voltage value increased the number of beads and effected to the shape of beads because of its difficulty for solution in the jet to diffuse and vaporize from the fibers surface before they reached at the collected plate. Thus, the SF-nanofibers became small diameter and beaded fiber. In the case of smooth fibers, the observation was found in increasing of the fiber diameters. The result of fiber diameter has been considered as the main parameter to influence the changing of Young's modulus of SF-nanofibrous scaffold. This contribution provided some characteristic of Thai silk fibroin nanofibers that could be the utility data for an applied biomaterials and tissue engineering applications.
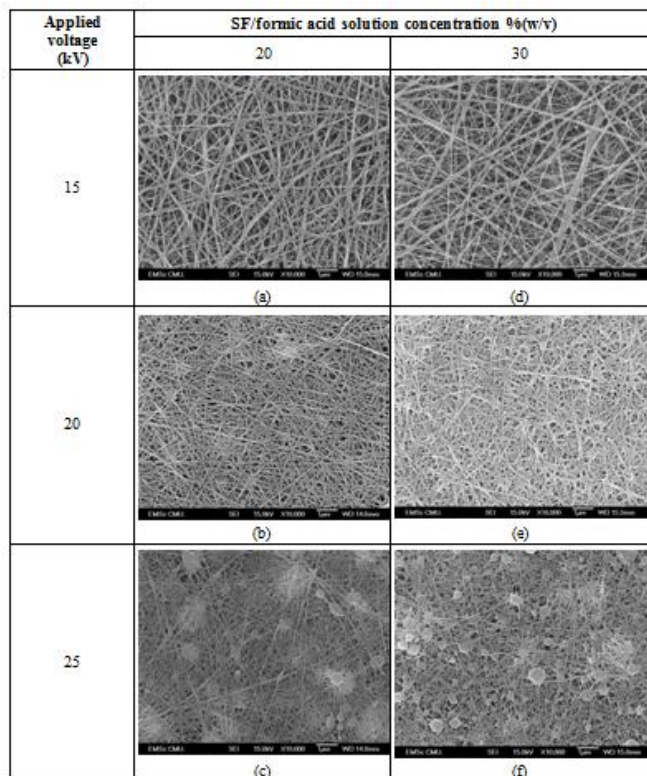

Figure 2. *FESEM images of electrospun SF-nanofibers and there corresponding processing parameters of electric field of 15, 20 and 25 kV and concentrations of 20 and 30% (w/v), respectively.*
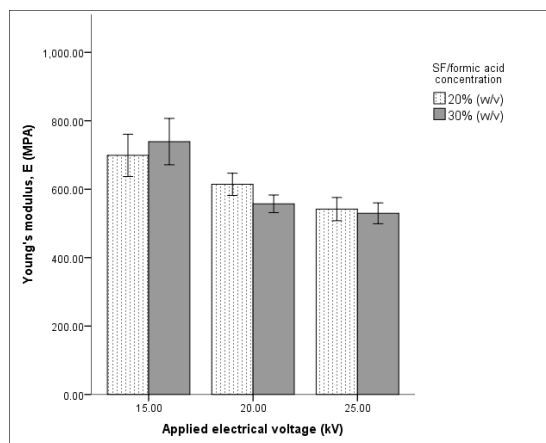

Figure 3. *Mechanical properties of electrospunThai silk fibroin nanofibrous scaffold.*

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