An Experimental study on Electrospinning of silk fibroin

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Abstract

Silk fibroin is one of candidate materials for biomedical application, because it has good biocompatibility and minimal inflammatory reaction. Electrospinning is a simple method capable of producing nano scale fibers from both synthetic and natural polymers for biomedical applications. In this study nonwoven matrices of silk fibroin (SF) nanofibers were prepared by electrospinning of regenerated SF solution. Effects of electric field and silk concentration in formic acid on the nanofibers uniformity, morphology and diameter were studied. Statistical analysis showed that the silk concentration was the most important parameter in producing uniform cylindrical fibers less than 400 nm in diameter. For insolubilization, as-spun SF nanofibers nonwovens were chemically treated with an aqueous methanol and ethanol solution and secondary structure of silk fibroin were determined using FTIR spectroscopy. It was shown that chemical treatment of the nonwovens increased ß-sheet content of the silk fibroin. Scanning electronic microscope (SEM) was used to observe the morphology of the fibers.

Keywords: electrospinning-nanofiber-silk- morphology-FTIR

1. Introduction

Natural fibers such as wool, cotton and silk are known as biocompatible and biodegradable materials. The physical properties of silks distinguish them from all other natural materials known to date. Most importantly, silks are unique for their combined extensibility and high tensile strength [1, 2, 3]. Silk proteins have a very large average molecular weight [4]. They are hydrophobic in their solid state, and in some cases their mechanical properties are greatly influenced by water [5,6]. Silks are thermally stable at very low as well as very high temperatures [7,8,9,10].

Among the native silk proteins, the silkworm silk, mostly that of the domesticated Bombyx mori, has been accepted as a high quality textile fiber and suture for a long time [11]. B. mori silk fiber has been shown to be composed of two protein-monofilaments (named brins) embedded in a glue-like sericin coating [12,13]. A similar structure has been observed in other silkworms silk [14]. The brins are fibroin filaments made up of bundles of nanofibrils, approx 5 nm in diameter, with a bundle diameter of around 100 nm [12,15]. The brins are fibroin filaments made up of bundles of nanofibrils, approx 5 nm in diameter, with a bundle diameter of around 100 nm [12,15]. The nanofibrils are oriented parallel to the axis of the fibre, and are thought to interact strongly with each other [10,12]. Fibroin contains 46% glycine, 29% alanine and 12% serine. Fibroin is a giant molecule (4700 amino acids) comprising a “crystalline” portion interacts strongly with each other [10,12]. Fibroin contains 46% glycine, 29% alanine and 12% serine. The crystalline portion comprises about 50 repeats of polypeptide of 59 amino acids whose sequence is known:

Gly-Ala-Gly-Ala-Gly-Scr-Gly-Ala-Ala-Gly-(Scr-Gly-Ala-Gly-Ala-Gly)s-Tyr This repeated unit forms, β - sheet and is responsible for the mechanical properties of the fiber[16]. The sericin coating constitutes 25–30% of the weight of B. mori’s silk fiber, and helps in the formation of silk cocoon by gluing the fibers together [10,17,18]. Sericin contains 37% serin, 17% glycin and 16% aspanate[16]. Silk fibers have proven to be effective in many clinical applications during decades of use, but on the other hand some biological responses to the protein have raised questions about biocompatibility such as inflammation and degradation. However, recent studies demonstrated that the sericin glue-like proteins are the major cause of adverse problems and the biological responses to the fibroin fibers appear to be improved, if sericin is removed [1,19,20,21,22].

In recent years, the electrospinning process has gained much attention because it is an effective method to manufacture ultra fine fibers or fibrous structures of many polymers with diameter in the range from several micrometers down to tens of nanometers [23-25] from a polymer solution a charged jet is created, when the electrical force overcomes surface tension. The jet typically develops a bending instability and then solidifies to form fibers, which measures in the range of nanometers to 1 μm. Most studies so far have been done with polymer solutions that had always been used as fibers, such as poly (ethylene oxide), poly (ethylene terephethalate) and others.

Recently, many researchers have investigated silk based nanofibers as one of the candidate materials for biomedical applications, because it has several distinctive biological properties including good biocompatibility, good oxygen and water vapor permeability, biodegradability, and minimal inflammatory reaction [20,26,27] .In practice, SF has been used in various fields, such as cosmetics, medical materials for human health, and food additives [23].

Electrospun SF nanofibers, as well as other types of SF matrices (e.g., film), often require chemical treatments with aqueous alcohol solutions in order to enhance their stability and mechanical properties. In this study, electrospun SF nanofibers were prepared and treated with solvents including methanol, ethanol, in order to construct stable nonwoven matrices. [28]

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2. Experimental

2.1 Materials

Silk fibers were from domestic suppliers. All other chemicals used were laboratory grade.

2.2 Preparation of regenerated SF solution

Raw silk fibers were degummed with 2 gr/L Na2CO3 solution and 10 gr/L anionic detergent at 100 ° C for 1 h and then rinsed with warm distilled water. Degummed silk (SF) was dissolved in a ternary solvent system of CaCl2/CH3CH2OH/H2O (1:2:8 in molar ratio) at 70 ° C for 6 h. After dialysis with cellulose tubular membrane (Bialysis Tubing D9527 Sigma) in H2O for 3 days, the SF solution was filtered and lyophilized to obtain the regenerated SF sponges.

2.3 Preparation of the spinning solution

SF solutions were prepared by dissolving the regenerated SF sponges in 98% formic acid for 30 min. Concentrations of SF solutions for electrospinning was in the range from 8% to 14% by weight.

2.4 Electrospinning

In the electrospinning process, a high electric potential (Gamma High voltage) was applied to a droplet of SF solution at the tip (0.7 mm in external diameter) of a syringe needle, The electrospun nanofibers were collected on a target plate which was placed at a distance of 10 cm from the syringe tip. A high voltage in the range from 10 kV to 20 kV was applied to the droplet of SF solution at the tip.

2.5 Solvent treatments

Electrospun SF nanofiber matrices were treated with solvent, including methanol, and ethanol, in order to achieve solvent-induced crystallization of fibroin. Briefly, solvent vapor-treated samples were prepared by placing SF nanofiber matrices in palates saturated with solvent for 1 h and then dried in air at room temperature for 2 h.

2.6 Characterization

Scanning electron microscope (SEM, Philips XL-30) was used to investigate the macroscopic morphology of electrospun SF fibers. Fourier Transform Infrared Spectroscope (Nicolet magna 560) was used to elucidate the structure of the silk fibroin in the electrospun fibers.

3. Results and discussion

3.1 Effect of silk concentration

On the base of our experiments electrospinning of solution the silk concentration of 8% was not successful. When concentration of the silk solution was increased to 8%, nanofibers were formed at high electric field (20 KV). Below the silk concentration of 8% as well as at low electric filed in the case of 8% solution, droplets were formed instead of fibers. Fig. 1 shows morphology of the obtained fibers from 8% silk solution at 20 KV. The obtained fibers are not uniform and branched off. The average fiber diameter is 80 nm and a narrow distribution of fiber diameters is observed. It was found that continues nanofibers were formed above silk concentration of 8% regardless of the applied electric field and electrospinning condition. A series of experiments were carried out when the silk concentration was varied from 8 to 14% at the 15KV constant electric field. Figs 2-4 show the SEM micrographs and diameter distribution of the resulted fibers. In these cases thin and rod like fibers with diameters range from 80-500 nm were obtained. Fig. 5 shows the relationship between mean fiber diameter and silk concentration at the electric field of 15 KV. There is a significant increase in mean fiber diameter with the increasing of the silk concentration, which shows the important role of silk concentration in fiber formation during electrospinning process. Concentration of the polymer solution reflects the number of entanglements of polymer chains in the solution, thus solution viscosity. Experimental observations in electrospinning confirm that for fiber formation to occur, a minimum polymer concentration is required. Below this critical concentration application of electric field to a polymer solution results electrospraying and formation of droplets to the instability of the ejected jet. As the polymer concentration increased, a mixture of beads and fibers is formed. Further increase in concentration results in formation of continuous fibers as reported in this paper. It seems that the critical concentration of the silk solution in formic acid for the formation of continuous silk fibers is 10%.

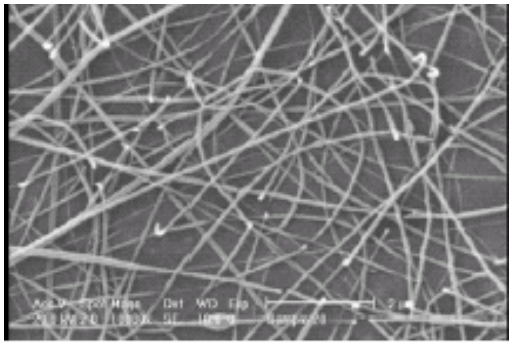


Fig.1. SEM micrograph and fiber distribution of 8wt% of silk at 20 KV

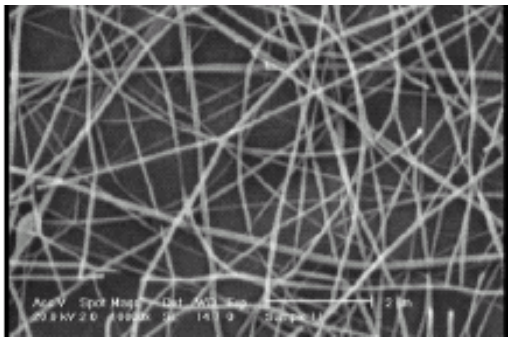


Fig.2.SEM micrograph and fiber distribution of 10wt% of silk at 15 KV

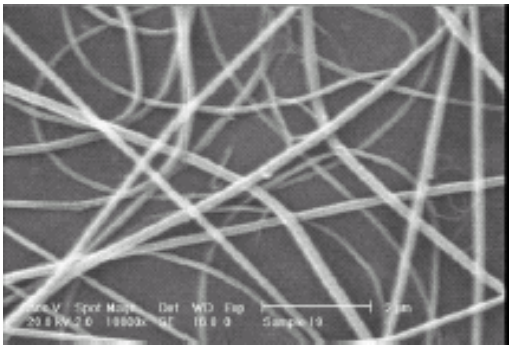
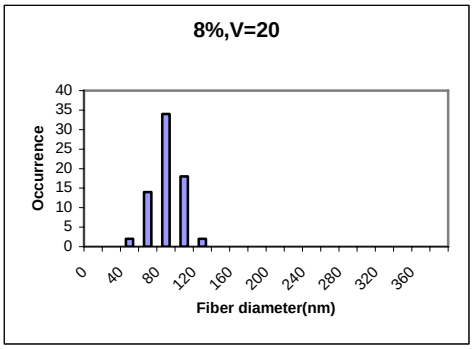
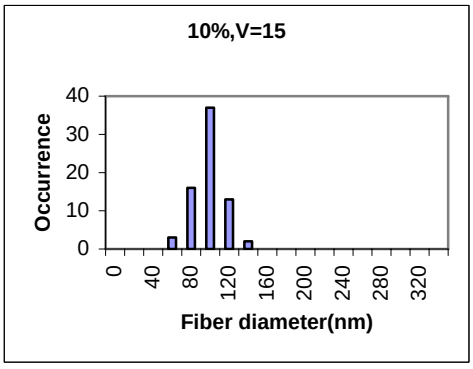
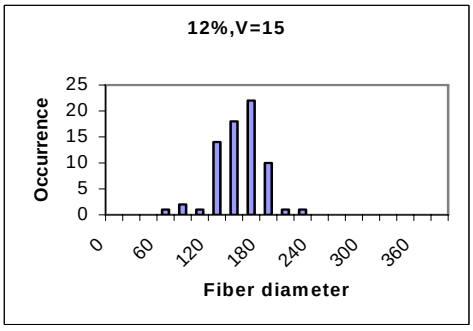


Fig.3.SEM micrograph and fiber distribution of 12wt% of silk at 15 KV







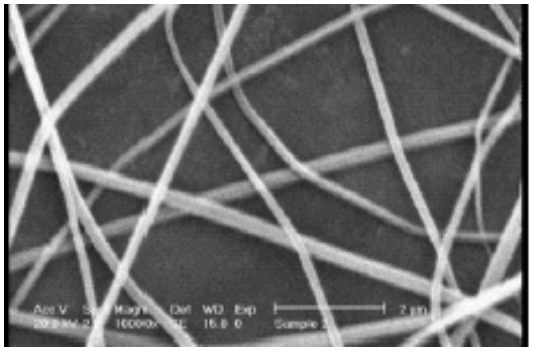


Fig.4.SEM micrograph and fiber distribution of 14wt% of silk at 15 KV

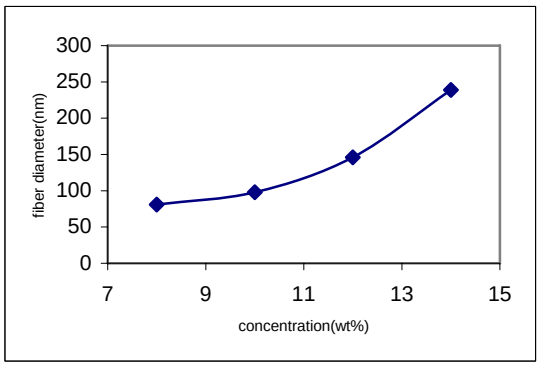


Fig.5.mean fiber diameter of electrospun silk fibers at 15 KV

3.2 Effect of electric field

It was already reported that diameter of electrospun fibers was insignificantly affected by the applied voltage. In order to study the effect of the electric field, silk solution with the concentration of 12% was electrospun at 10, 15, and 20 KV. Figs. 6,3,7 show SEM micrographs and fiber diameter distribution of the resulted fibers at 10, 15 and 20 KV, respectively. It is clearly seen that at all electric fields continuous and uniform fibers are formed but the fiber diameter distribution is narrowest at 10 KV (lower electric field). The variation of the mean fiber diameter at different applied voltage is shown in Fig. 8. The results suggest a slight and insignificant increase in the mean fiber diameter with the increasing of the electric fields for the 12% silk solution. It is suggested that, higher applied voltage causes multiple jets formation, which would provide non-uniform fiber diameter and broad fiber diameter distribution.

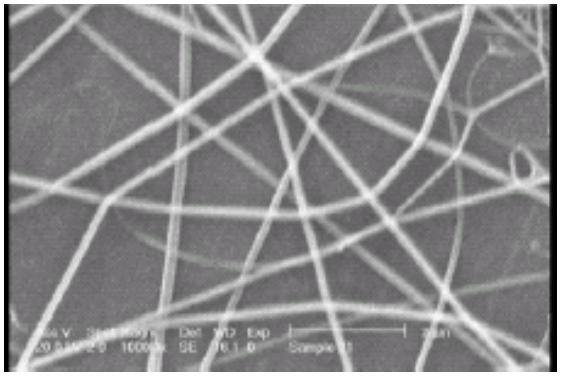


Fig.6.SEM micrograph and fiber distribution of 12wt% of silk at 10 KV

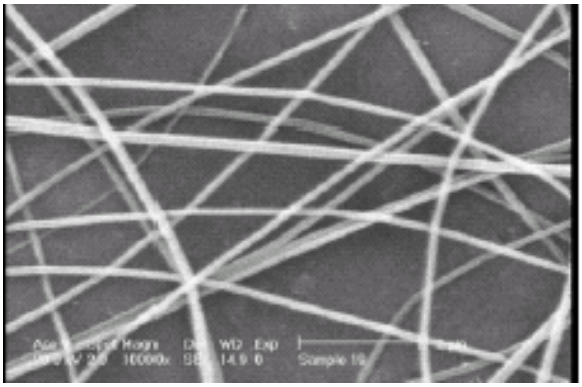
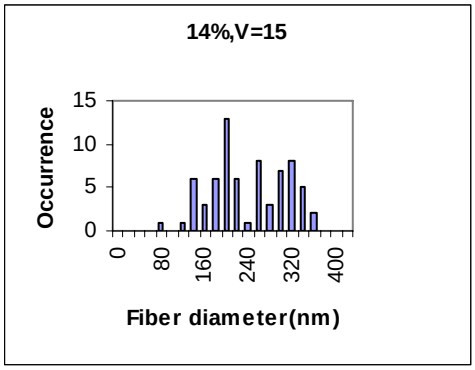
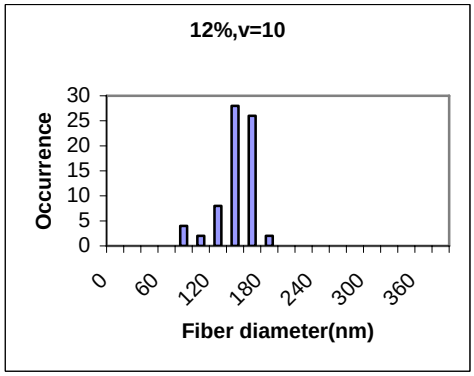


Fig.7.SEM micrograph and fiber distribution of 12wt% of silk at 20 KV





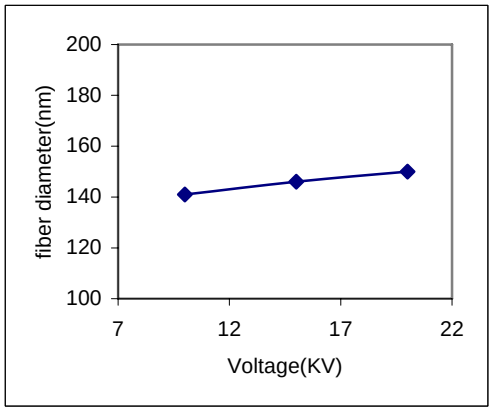


Fig.8.Mean fiber diameter of electrospun silk fibers at concentration of 12%

3.3 FTIR spectroscopy

FTIR spectroscopy is used to investigate the performance of SF and its blends because the IR spectrum represents typical absorption bands sensitive to the molecular conformation of SF. SF exist in α-helix or β-sheet conformation depending on preparation condition. Each conformation has characteristic absorption bands on FTIR spectrum. The bands at 1630, 1530 and 1265 cm-1 are assigned to β-sheet conformation while the bands at 1660, 1540 and 1235 cm-1 are assigned with α-helix conformation. Fig. 9 shows the FTIR spectra of as spun, methanol and ethanol treated silk nanofiber mats. As spun nanofiber mat shows characteristic bands at 1650, 1540 and 1242 cm-1, which implies α- helix conformation for SF. These characteristic bands for methanol and ethanol treated mats have shifted to 1629, 1521 and 1265 cm-1, which are characteristic bands of β-sheet conformational structure. Therefore treatment of as spun silk nanofiber mats with methanol and ethanol will change the structural the structural conformation of the mats from α- helix conformation to β-sheet conformation.

4. Conclusion

The electrospinning of silk fibroin in formic acid was successful and fibers with diameters ranging from 80 to 500 nm were obtained depending on electrospinning conditions. The concentration of silk solution played an important role on fiber formation and it was found that 10% concentration of silk solution was the lower critical concentration limit for fiber formation in electrospinning process. Variation of applied electric field had not a significant effect on the mean fiber diameter but the narrowest fiber diameter distribution was obtained at lower electric field.

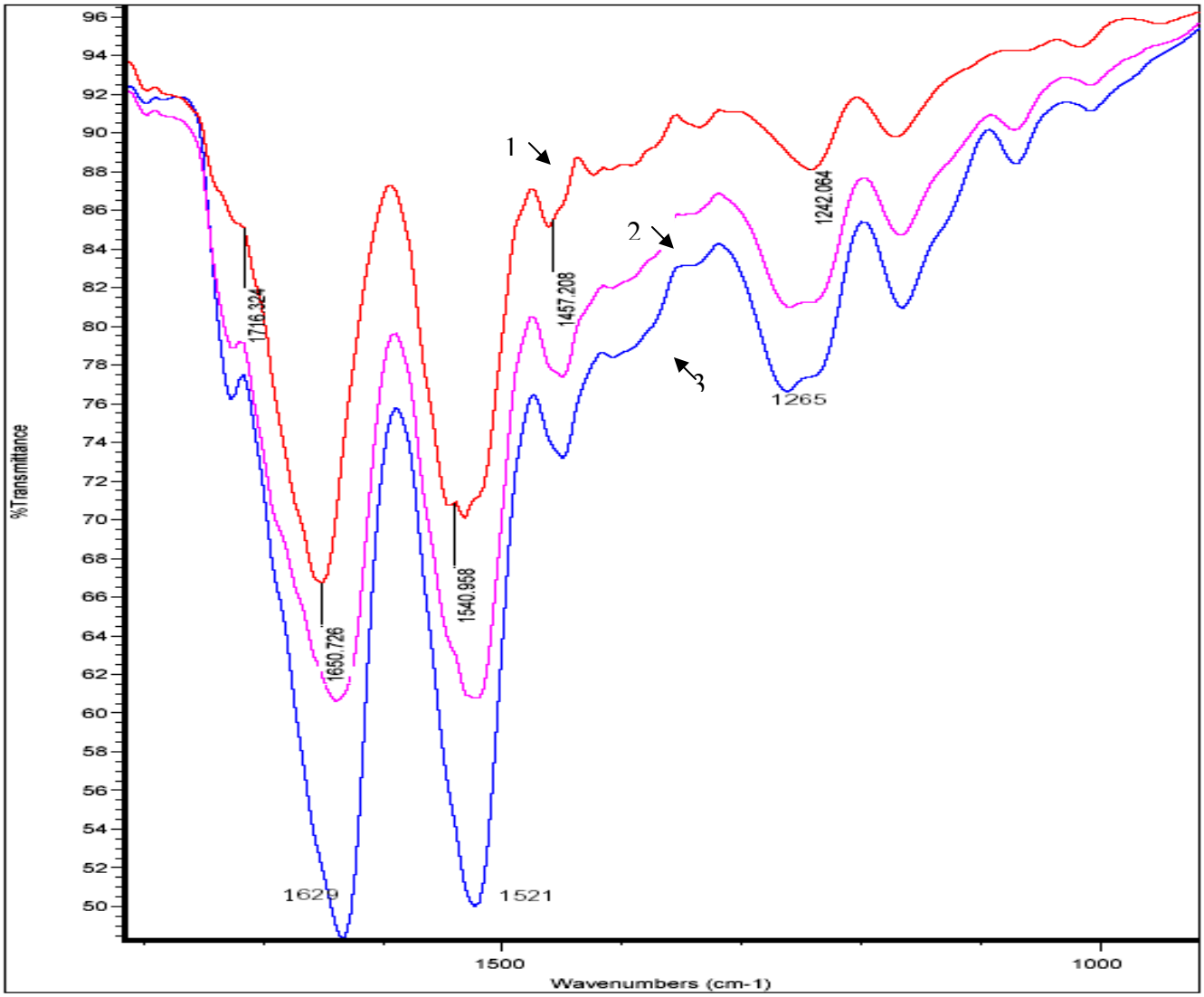


Fig.9- FTIR Spectra of (1) electrospun silk nanofibers mat, (2) methanol treated mat and (3) ethanol treated mat

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