International Journal of Biological Macromolecules 87 (2016) 201–207

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

A novel electrospinning approach to fabricate high strength aqueous silk fibroin nanofibers

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ARTICLE INFO

Article history:
Received 30 October 2015
Received in revised form 28 January 2016
Accepted 30 January 2016
Available online 18 February 2016

Keywords:
Regenerated silk fibroin
β-Sheet content
Conformational transition
Electrospinning

ABSTRACT

The present paper describes a rapid method of producing concentrated aqueous regenerated *Bombyx mori* silk fibroin (RSF) solution by applying mild shearing under forced dehumidified air and generation of electrospun SF nanofibers from concentrated solution with high mechanical strength using free liquid surface electrospinning machine. The shear induced concentrating mechanism favoured the electrospinning process by enhancing the viscosity (>2.43 Pa s as onset for electrospinning) and decreasing the surface tension of the solution (40.1–37.7 mN/m). Shearing reduced the β-turns and random coil molecular conformation and thereby, intensified the β-sheet content from 16.9% to 34% which is the minimum content needed to commence RSF nanofibers formation. Subsequently, electrospun nanofibrous mats were produced from different batches of concentrated SF solutions (15–21 wt%). Among the concentrated RSF, 17 wt% RSF solution was the most favourable concentration producing electrospun nanofibrous mat having lowest average fiber diameters of 183 ± 55 nm and good tensile strength. The mechanical strength of the nanofibrous sheet was further improved by cross-linking with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and *N*-hydroxysuccinimide (EDC + NHS) which might be due to enhancement of β-sheet content. These nanofibers exhibited 17.57 ± 1.13 MPa ultimate tensile strength, 12.48 ± 1.46% tensile strain at break and 37.7% increase in root mean square surface roughness which is favourable feature for cell adhesion and neo-tissue formation.

1. Introduction

Silk fibroin (SF) derived from *Bombyx mori* (*B. mori*) silk cocoon has been used by the researchers worldwide as a promising biomaterial for various applications such as in drug delivery, wound dressing and very recently in the development of organ and tissue constructs [1,2]. The unique properties such as biocompatibility, biodegradability, mechanical strength and bioactivity made SF as an excellent biomaterial for developing tissue engineered scaffolds [2–4]. In tissue engineering, the fabrication of three-dimensional (3D) porous matrices that mimic the structure and function of body extracellular matrix is our prime goal. In this context, SF nanofibers generated by electrospinning method is more advantageous than the other forms of SF such as film, 3D porous structures etc because of their high surface area, high porosity and provide favourable microstructure for cell adhesion, proliferation and neo tissue regeneration [5–7].

Electrospun *B. mori* SF nanofibers have been previously prepared using a variety of organic solvent namely formic acid, hexafluoro-2-propanol (HFIP), hexafluoroacetone, trifluoro acetic acid [8–11]. The use of these spinning solvents cause many adverse effects like spinnability of polymer solution, their surface morphology and mechanical strength which are crucial for *in-vivo* applications [12]. Furthermore, the harsh effects of organic solvents induce corrosive and catalytic actions causing the deposition of corrugated nanofibers over the collector, which further reduce the mechanical strength of the scaffold. To overcome such drawbacks of using organic solvent, aqueous solvents are considered appropriate because of their low toxicity, less interference in natural structures, controlled degradability and their ability to impart a plasticizing property to the nanofibers thereby increased mechanical strength [13].

To this end, recently processes generating aqueous electrospun SF based nanofibers with high mechanical strength without using harmful organic solvent was reported since the first study performed by Jin et al. [14,15]. All these studies has provided scope for further improvement in the process or developing new method to produce aqueous SF solution with high concentration and high mechanical strength for the wider application of the this poten-

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http://dx.doi.org/10.1016/j.ijbiomac.2016.01.120
0141-8130/

tial biomaterial [15–17]. However, some or other way, all these process have their own advantages and limitations as for example higher fiber diameter, unpredictable physico-chemical properties, time consuming to prepare higher concentration solution, lower mechanical strength, gel formation etc and thus provides scope for further study to address these issues [17–20].

Therefore, the present research aimed to devise a novel and rapid method of preparing aqueous RSF solution with high concentration by applying shearing under forced dehumidified air and generation of eletrospun SF nanofiber with high mechanical strength using free liquid surface electrospinning machine. The choice of free liquid surface electrospinning method is advantageous because of rapid fabrication of nanofiber with higher surface area to volume ratio without any inherent/choking problem associated with conventional needle based electrospinning method. Furthermore, particularly in hard tissue regeneration, enhancing the mechanical strength of SF nanofiber is a major prerequisite. To achieve further improvement in mechanical strength, besides the use of aqueous solvent, the electrospun nanofiber was cross-linked with EDC + NHS as cross-linking agent [21].

2. Materials and methods

2.1. Materials

B. mori cocoons were purchased from Central Tasar Research & Training Institute (Jharkhand, India). Lithium bromide (LiBr) and Sodium carbonate (Na_2CO_3) was obtained from Sigma–Aldrich (USA). The dialysis cassettes were bought from Thermo fisher (USA).

2.2. Preparation of aqueous RSF solution

Silk fibroin was extracted from *B. mori* silk cocoons following the literature published earlier [22,23]. Then the silk fibers were washed thoroughly to remove sericin and dried at 37 °C overnight. The degummed SF was dissolved in 9.3 M LiBr aqueous solution at 45 °C to prepare a 10% (w/v) solution. The obtained solution was transferred carefully into the dialysis cassette and dialyzed against deionized water for 2–3 days. Followed by, centrifugation of obtained SF solution at 9000 rpm to remove suspended agglomerates. The concentration of dialysed regenerated silk fibroin solution was determined [23]. The prepared RSF solution was concentrated by using a cylindrical spinning electrode that constitutes a part of the electrospinning machine under forced dehumidification (110 CFM) with varying shear rates of 4, 8 and 12 rpm of cylindrical electrode at 20 ± 2 °C.

2.3. Generation of SF nanofibers by electrospinning method

The prepared concentrated RSF solution was used for the generation of nanofibers using a free liquid surface electrospinning machine (Elmarco NS Lab 200, Czech Republic). The electrospinnability of the concentrated RSF solution was checked by applying high electric field (3.2 kV/cm) between the spinning electrode (16 cm in length, 2 cm in width) and collector placed 12–18 cm apart for proper fiber deposition at 20 ± 2 °C. In this technique, when the applied electric force overcomes the surface tension of the SF solution, Taylor cone forms and causes the development of charged jets which eject in the direction of the applied field to produce nanofibers. These nanofibers were then collected in the form of a mat and cross-linked with 3 wt% EDC + NHS [2:1 (w/w)] in (95:5 v/v) ethanol:water solution. The residual cross-linking reagent was removed by rinsing in deionized water and dried in an oven at 40 °C for 24 h.

2.4. Characterizations

2.4.1. Characterization of RSF solution

The viscosity of the prepared RSF solution was measured by Bohlin Visco-88 (Malvern, UK) viscometer in linear increments of shear rate from 0.1 to 100 s^{-1} at 25 °C. The surface tension of the polymer solution was determined through KRUSS Tensiometer (K100) by following ring method [24]. The maximum force experienced while stretching was recorded and the surface tension was calculated by the equation: $\sigma = F_{max}/(L \times \cos\theta)$, where σ = surface tension, L = wetted length, F_{max} = maximum force and θ = contact angle.

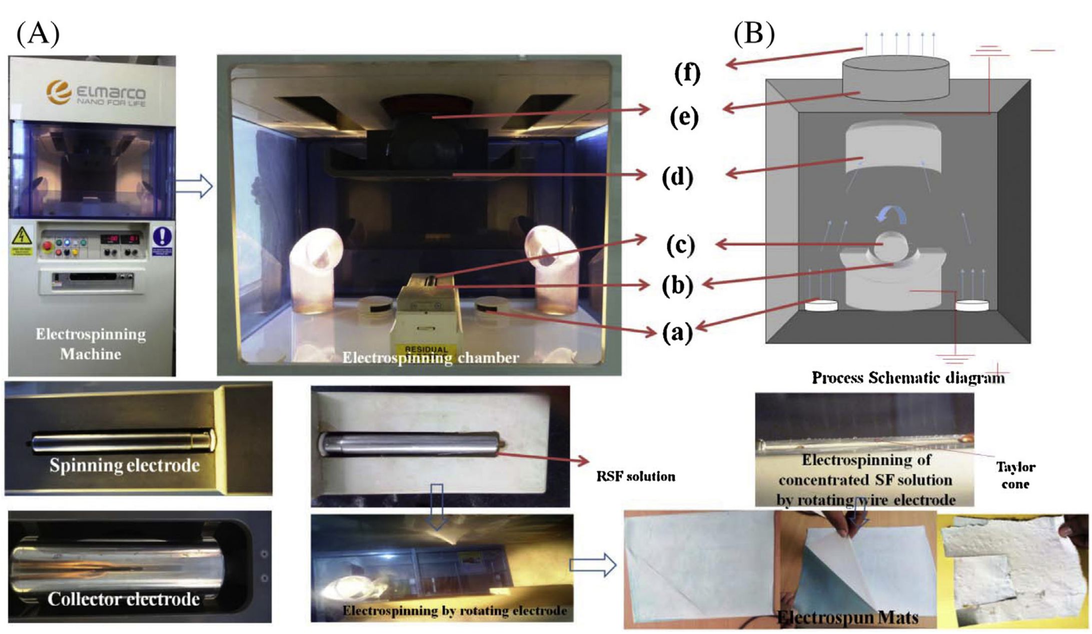

Fig. 1. (A) Free liquid surface electrospinning machine used for generation of electrospun nanofibers. (B) The schematic device for concentrating RSF solutions (a) dehumidified air, (b) polymer solution in tray, (c) spinning electrode, (d) collector, (e) suction fan and (f) water vapour and air.