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Insights into the production and characterization of electrospun fibers from regenerated silk fibroin

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ABSTRACT

Regenerated silk fibroin solutions from *Bombyx mori* were tested for electrospinning. Simple and reproducible tensile tests were performed on threads of aligned fibers to obtain information about their mechanical performance at the fiber level. The binary solvent formic acid/chloroform (10:1, v/v) rendered unbeaded thinner fibers with increased extensibility before failure when compared with pure formic acid. A remarkable improvement in strength was induced by immersing length-restricted fibers into ethanol for 5 min. Conformational changes of the protein chains were studied by solid-state NMR.

1. Introduction

Regenerated silk fibroin (SF) from *Bombyx mori* has been proven to be fully biocompatible [1] and can be processed in different formats (films, 3D-sponges, hydrogels, nanofibers, etc.), which makes it possible to tune their degradability rate [2,3]. On the other hand, the morphology of a biomaterial greatly influences its bio-application, and porous architectures composed of nanofibers that could mimic the native nanofibrous extracellular matrix (ECM) [4,5] are especially promising for tissue regeneration [6–8].

Electrospinning is an attractive method of producing nanoscale fibers from both natural [9–13] and synthetic polymers [14–18] with diameters ranging from tens of nanometers to few micrometers. Electrospinning of silk was first reported by Zarkoob et al., who used hexafluoro-2-propanol (HFIP) to dissolve native silk from both *Nephila clavipes* and *B. mori* [19]. Solvent type greatly influences the electrospinning behavior of the regenerated fibroin solution, but other experimental parameters such as molecular weight distribution and chemistry of the constituent proteins, concentration and pH of the solution, voltage, spinning distance, relative humidity and temperature also have their effect on the spinning process and thus on the properties of the obtained fibers [10,20–44].

Different techniques have been used to characterize the electrospun materials in terms of fiber morphology, size and porosity, but also structural features such as

Abbreviations: CP-MAS, cross-polarization magic-angle spinning; FA, formic acid; HFIP, hexafluoro-2-propanol; I.D., inner diameter; MWCO, molecular weight cut-off; NMR, nuclear magnetic resonance; PEG, polyethylene glycol; PEO, polyethylene oxide; SEM, scanning electron microscopy; SF, silk fibroin; XRD, X-ray diffraction.

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crystallinity and molecular conformation have been studied [21–34,36]. However, the mechanical properties of the electrospun fibroin materials produced have been analyzed only in some cases [21–24,30–33]. In addition, due to experimental difficulties and limitations, the tensile tests have been performed mostly on electrospun fiber mats rather than on single fibers or aligned yarns of fibers [27]. An appropriate methodology to obtain and analyze electrospun aligned fibers is needed, as they offer the potential to mimic oriented tissue architecture as the one found in ligament or muscle tissue [13,37,38].

In this work we addressed both the morphological and mechanical characterization of electrospun threads of aligned fibers, which were further correlated with our NMR structural studies. For this purpose, we used regenerated silk fibroin (SF) and explored both aqueous and non-aqueous solvents to prepare the spinning dopes.

In addition, we wanted to analyze the influence that the introduction of subtle changes in the electrospinning solution might have on the properties of both aligned yarns and non-woven fiber mats. We particularly focused on solvent composition and compared pure formic acid (FA) with a mixture FA/chloroform (10:1 v/v). Finally, the effect of a very short ethanol immersion as post-spinning treatment was also studied at both the macroscopic and molecular levels.

2. Experimental section

2.1. Preparation of spinning dopes

B. mori silkworm silk cocoons were kindly supplied by Dr. Silvia Cappellozza from *Consiglio per la Ricerca e la Sperimentazione in Agricoltura*, Bologna (Italy). Cocoons were cut into small pieces and impurities were discarded. Sericin was removed by two repeated boiling treatments in aqueous 0.5% (w/v) Na_2CO_3 for 30 min, each followed by thorough rinse with deionized water at 80 °C for 10 min. Degummed silk was then allowed to dry at room temperature for 24–48 h and dissolved in aqueous 9.4 M LiBr. SF solution was next dialyzed against aqueous 20% (w/v) polyethylene glycol (PEG) for 3 days (*Fisher scientific*, Slide-A-Lyzer Dialysis Cassette; MWCO 3500), rendering a ~20 wt% fibroin concentration. SF aqueous solutions ranging 5–30 wt% were used for electrospinning. In some cases, 5–10 wt% of FA was added to the dope solution prior spinning. Alternative non-aqueous spinning dopes were prepared from lyophilized powder after freeze-drying of freshly dialyzed SF solution. Solid SF was dissolved in pure FA or in a mixture FA/chloroform (10:1 v/v) at room temperature, to obtain 18 and 17 wt% silk solutions, respectively. Continuous stirring was applied for 24 h to ensure complete dissolution.

2.2. Electrospinning

Each fibroin solution was fed into a 3-mL polypropylene syringe fitted to a 22-gauge stainless-steel needle (0.7 mm I.D.) and mounted on an electrically controlled pump (KD Scientific). The steel capillary tube was maintained at high electric potential using a high voltage power supply (*NANON-01A*, Mechanics Electronic Computer Corporation Ltd., Japan). Non-woven mats were deposited on an aluminum foil plate used as a stationary collector, while a rotating disk (Ø 25 cm; 1500 rpm) was employed to obtain aligned yarns of fibers. The distance between the spinneret and the collector was 10–20 cm, the flow rate of the feedstock was 0.2–1.0 mL/h and the applied voltage was 5–20 kV.

Non-woven mats and aligned fibers obtained from 18 wt% SF solutions in pure FA and spun at voltage of 15 kV, distance of 15 cm, and 1 mL/min flow rate are termed **S1**. Analogous samples electrospun at identical experimental conditions (i.e. 15 kV, 15 cm, 1 mL/min) from 17 wt% SF dopes in FA/chloroform 10:1 (v/v) will hereinafter be referred to as **S2**.

In some cases, as-spun non-woven mats were further immersed in pure ethanol for 5 min and let them dry overnight. Aligned threads of fibers were also subjected to this post-spinning step with or without length restriction (see Section 2.3.4). Samples obtained from **S1** and **S2** after the alcohol post-treatment will be referred to as **S1-E** and **S2-E**, respectively.

2.3. Characterization of electrospun samples

2.3.1. Morphological characterization

The morphology of nanofibrous mats was analyzed by Scanning Electron Microscopy (*JEOL JSM 6300*) with an automated pressure regulation system. A low accelerating voltage (10 kV) was used for imaging after coating samples with gold. Fiber diameters were determined by measuring ~100–200 fibers randomly selected from each electrospun material using *ImageJ* software [45].

2.3.2. Wide angle X-ray diffraction (WAXD) analysis

WAXD patterns were recorded by a *Philips X'Pert-MPD* (EQ 31-02) diffractometer operating at 45 kV and 40 mA with a *Cu* K_α radiation (λ = 1.54 Å). All the experiments were conducted at room temperature in the reflection mode. A continuous scan rate of 0.040°/min was applied within the scanning region of 2θ = 5°–35°.

2.3.3. Solid-state NMR structural study

^{13}C cross-polarization magic-angle spinning (CP-MAS) NMR experiments were carried out on a *Bruker Advanced 400 MHz Wide Bore* (9.39 T) spectrometer operating at 100.6 MHz for ^{13}C resonance. Samples were spun at 12 kHz (MAS) in a 4 mm-diameter ZrO_2 rotor at room temperature. Spectra were acquired with a ramp CP contact time of 2 ms, 1H 90° pulse width of 2.5 μs, recycle delay of 5 s and acquisition time of 50 ms. A total of 5000–12000 scans were collected over a spectral width of 28.3 kHz. TPPM15 1H decoupling with field strength of 62 kHz was applied during signal acquisition. Chemical shifts were reported relative to external adamantine (29.5 ppm). For each sample, NMR Ala Cβ peak was deconvoluted into Gaussian functions using *Fityk* software in order to analyze quantitatively each secondary structure.