Conductive regenerated silk fibroin composite fiber containing MWNTs

Negin Piri, Vahid Mottaghitalab,∗ Shahram Arbab

∗Textile Engineering Department, Faculty of Engineering, P.O.Box 3756, University of Guilan, Rasht, Guilan, Iran; fax: 0098-1316690270; e-mail: motaghitalab@guilan.ac.ir

(Received: 22 May, 2012; published: 24 February, 2013)

Abstract: The electrically conductive regenerated silk fibroin (RSF) fiber containing multiwalled carbon nanotubes (MWNTs) was developed through wet spinning process. A general scheme used for extraction of RSF powder as biopolymer matrix for MWNTs conductive filler. The MWNTs inclusion demands for a sophisticated approach to prepare stable, exfoliated and well distributed filler in matrix solution. Dynamic Light Scattering (DLS) was utilized to show the ability of RSF in stabilizing of MWNTs throughout spinning solution. A non-Newtonian shear thinning behavior observed for RSF/MWNTs according to strong chain entanglement, whereas, the neat RSF solution shows a lower dependence on the shear rate. The scanning electron microscopy (SEM) images also confirmed the proper distribution of MWNTs as a continuous fibril media in the RSF matrix. The Fourier transform infrared spectroscopy (FTIR) demonstrated the role of MWNTs inclusion for increasing of the crystalinity compared to bare RSF fiber. The Raman spectra also confirmed the domination of RSF signatures by strong Raman bands of MWNTs most specifically in RBM region regarding to superior nanotube exfoliation. Moreover, the measurement of four-point probe electrical conductivity for different MWNTs content shows a maximum conductivity of 0.12 S/cm at percolation threshold. The higher thermal stability and greater crystalinity were also evidenced by DSC thermal analysis.

Introduction

The regenerated silk fibroin (RSF) derived from natural silk has been exploited as a highly promising biopolymer with a variety of biomedical applications [1]. The extracted virgin silk (VS) from the cocoon of β. mori contains at least two major fibroin proteins including low and high molecular weight respectively 25 and 325 kDa. The two core fibroin fibers are encased in a sericin coat composed of a family of glue-like proteins [2, 3]. The RSF is a large macromolecule containing both crystalline and amorphous regions. From structural point of view, silk fibroin is known as a natural block copolymer in which hydrophobic blocks contain amino acids with short side chains and hydrophilic blocks contain amino acids with long side chains and charged amino residues [4-5]. Despite centuries of use as textile material, silk matrices are now being rediscovered and reconsidered as potentially useful biomaterials for a range of applications in clinical regenerative medicine and in vitro as scaffolds for tissue engineering [6-10]. The RSF is a biocompatible and slowly biodegradable biomaterial with excellent mechanical properties that could meet the basic required properties for nerve conduits [6], drug control release [7,8], artificial vein implants [9] and skin repair [9]. Moreover, RSF fibers show proper mechanical strength and there is no evidence for its selected interaction in human body.
Regarding to these behaviors, RSF matrices are potentially useful for bone cell growth [10]. The RSF-based nerve graft (RSF graft) which was composed of a RSF-nerve guidance conduit (NGC) inserted with oriented RSF filaments prepared via well-established procedures exhibits an eggshell-like microstructure that is responsible for its superior mechanical and permeable properties beneficial to nerve regeneration [11,12]. Currently, efforts are aimed at adjusting the structure of biomaterials to their required functions in order to address a broad range of biomedical needs. In this context, methods to extract RSF have been developed, and several silk based biomaterials, such as silk porous scaffolds [6], silk fibers [13, 14] and films [15] and electrospun nanofibers [16], with the inclusion of new useful functionalities can be processed from silk solutions. These productions can have ameliorated by blending RSF with other polymers and multifunctional nanostructure additives [17]. Accordingly, the polymer nanocomposites, in which biopolymers serve as hosts for inorganic particles of nanoscale dimensions, have attracted scientific and technological interest [18]. Indeed, choice of carbon nanotubes (CNTs), with 1D structure and considerable nanochannels, is speculated to be excellent filler for RSF matrixes [19, 20]. The CNTs are one of the most important conductive macromolecules that were discovered by Iijima in 1991 [21]. Outstanding properties of CNTs make them useful materials in variety of applications such as sensors, actuators [22], nanomedicine [23]. Recent studies showed that MWNTs incorporated biopolymer scaffolds may be alternative materials for nerve regeneration and functional recovery in neural tissue engineering.[24, 25]. Moreover, the growth of various cells has been achieved using CNTs scaffolds. The acquired SEM images confirmed the adhesion of cellular neuron on CNT precursor [26,27].

Preparation of RSF filament have long been investigated but MWNTs incorporated spun RSF microfiber still needs to be addressed to find if nanotubes inclusion modify the general engineering characteristics of nanocomposite fiber based on RSF substrate for future cell growth study. Current study focuses on the development and characterization of electrically conductive RSF/MWNTs nanocomposite fibers.

Results and discussion

The rheological behavior of bare RSF and RSF/MWNTs spinning solutions has been investigated through viscosity measurement versus shear rate (Figure 1). The significant dependence of viscosity to nanotube loading is extremely clear based on given data. Also the data reproduced by meaningful error originated from nanotube distribution quality. At lower nanotube content, the negligible error was revealed. However the error moved up to higher level for samples with higher nanotube loading. It has been illustrated that the viscosity at low shear rate is a meaningful criterion for processes such as film casting, however, for continuous fibre spinning, the effect of high shear at the orifice of the spinneret should be taken into account. As the nanotube content increases in this composite system, nanotube–nanotube interaction and nanotube-RSF interactions begin to dominate producing a higher viscosity, particularly at low shear rate. In addition, solutions containing nanotubes showed a non-Newtonian shear thinning behavior, whereas, the neat RSF solution shows a lower dependence on the shear rate. The presence of MWNTs in the RSF solutions facilitated a greater entanglement of polymer chains through the strong interactions between the MWNTs and the RSF. Consequently, the viscosity at low shear rates was higher for solutions containing more MWNTs. High shear mixing likely disrupt MWNTs /RSF interactions, therefore reducing the viscosity. This
rheological behavior is quite consistent with trends observed for similar composite spinning solution containing a polymer matrix and single walled nanotubes (SWNTs). [28]

![Graph](image-url)

**Fig. 1.** The influence of MWNTs content on viscosity of RSF/MWNTs solution in different shear rate.

Dynamic Light Scattering (DLS) is a sophisticated and straightforward technique for acquisition of the size and size distribution data for small particles in dispersed form. In fact, it relates the rate of Brownian motion to particle size distribution. The hydrodynamic diameter (DH) of MWNTs bundles in RSF solution with RSF/MWNTs weight ratio of 0.5/1, demonstrates large bundles and unstable dispersion even in higher sonication time (Figures 2 solid line) with respect to polystyrene latex (Figures 2 dash line).
Fig. 2. The size distribution diagrams for a 0.5/1 RSF/CNT solution sonicated for different time compared to a 60 nm polystyrene latex. Figures 3 show the acquired DLS data at a 1/1 ratio of RSF/ MWNTs. The average D_H of MWNTs in RSF solution decreases by increasing RSF concentration, but yet, some levels of instability is detectable compared to polystyrene latex sample. The more stable solution, the smoother correlation diagram develops with faster relaxation time.

Fig. 3. The size distribution for a 1/1 RSF/MWNTs solution sonicated for different time compared to a 60 nm polystyrene latex.

Turning to third series of DLS test results (Figure 4), by increasing the RSF/ MWNT_S ratio to 2/1, the average D_H of MWNT_S bundles experienced a decrease to
approximately 200 nm and also stability of dispersion raised significantly with respect to standard sample.

![Graph showing size distribution](image)

**Fig. 4.** The size distribution diagrams for a 1/1 RSF/MWNTs solution sonicated for different time compared to a 60 nm polystyrene latex. According to the DLS data the average $D_H$ of MWNTs bundles decreases by increasing of the RSF amounts, which seems to be attributed to remarkable wrapping effect of RSF.

The stability of RSF/MWNTs dispersion over a long period is confirmed by solution stability test. The images presented in Figure 5 indicate neither sedimentation nor aggregation of the MWNTs bundles even after one-month stagnation.

![Images of dispersions](image)

**Fig. 5.** Stability of RSF/MWNTs dispersion (a) after sonication and (b) one month after sonication.
Fig. 6. Scanning electron micrographs of wet-spun nanocomposite fibers. (a) RSF scale bar 50 μm (b) RSF scale bar 500 nm (c) RSF/MWNTs scale bar 50 μm (d) RSF/MWNTs scale bar 500 nm.

Figures 6 illustrates the cross-section of RSF (Figures 6 a and b) and RSF/MWNTs (Figures 6 c and d) filaments wet spun into a methanol coagulant at 25 °C with low and high magnification.

The methanol gives a circular cross section with strong coagulation power with easy removal in air without a complicated washing process. Therefore, it can be concluded that methanol is a good coagulant for the preparation of wet-spun RSF filament. Figure 6.d shows a clear perspective of individual MWNTs wrapped by RSF.

Figure 7 compares the FT-IR spectra of natural silk fibroin and regenerated silk fibroin. There is no considerable difference according to enhanced peak of amide region between former and latter form of fibroin. It proves the original structure of silk protein is preserved during extraction process. Three conformations of silk fibroin including amide I, amide II and amide III can be identified from peaks at 1630 cm\(^{-1}\), 1515 cm\(^{-1}\) and 1260 cm\(^{-1}\) respectively. Glycine-Glycine and Alanine-Glycine which show the polypeptide sequence in protein, are other important peaks located at around (975-1015) cm\(^{-1}\).
Fig. 7. FT-IR spectra of natural silk fibroin and regenerated silk fibroin.

Figure 8 represents the FT-IR spectra of RSF and RSF/MWNTs wet spun filaments. The interaction between MWNTs and RSF could be understood through recognition of functional groups. According to FT-IR spectra, all the important peaks remained unchanged during the regeneration and spinning process. FT-IR spectroscopy of RSF shows two signals in the Amide I region at 1650 cm\(^{-1}\) and 1630 cm\(^{-1}\). Amide I peak at 1630 cm\(^{-1}\) represents β-sheet configuration whereas at 1650 cm\(^{-1}\) represents random coil and helical structure. The ratio of area under the curve for 1630 cm\(^{-1}\) to total area under the curves for 1630 cm\(^{-1}\) and 1650 cm\(^{-1}\) \([\text{Area}_{1630}/ (\text{Area}_{1650} + \text{Area}_{1630})] \times 100\) gives the percentage of β-sheets in the sample [16]. By comparing these ratios, qualitative and quantitative analysis can be carried out to justify the degree of crystallinity in fibroin structure.

Tab. 1. Comparison of the β-sheet content of natural RSF, RSF and RSF/MWNTs nanocomposite fiber.

<table>
<thead>
<tr>
<th>Fibroin type</th>
<th>([\text{Area}<em>{1630}/ (\text{Area}</em>{1650} + \text{Area}_{1630})] \times 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural fibroin fibers</td>
<td>81.58%</td>
</tr>
<tr>
<td>RSF powder</td>
<td>41.52%</td>
</tr>
<tr>
<td>Neat RSF biofibers (take up velocity=70 cm/min)</td>
<td>57.02%</td>
</tr>
<tr>
<td>RSF/MWNT biofibers (take up velocity=70 cm/min)</td>
<td>69.38%</td>
</tr>
<tr>
<td>RSF/MWCNT biofibers (take up velocity=140 cm/min)</td>
<td>73.10%</td>
</tr>
<tr>
<td>RSF/MWCNT biofibers (take up velocity=210 cm/min)</td>
<td>73.62%</td>
</tr>
</tbody>
</table>
For quantitative analysis of the β-conformation, the content of the β-sheet structure, known as crystalline index, was determined and summarized in Table 1.

![Image](image_url)

**Fig. 8.** FTIR spectra of (a) Natural fibroin filament (b) RSF fibers (140 cm/min) (c) RSF/MWNTs (70 cm/min) (d) RSF/MWNTs (140 cm/min) (e) RSF/MWNTs (210 cm/min)

With respect to the FTIR analysis, Crystallization likely occurred after the passage of as spun fibers into the coagulation bath containing methanol. This coagulant is able to induce RSF crystallization by random-coil to β-sheet conformational transition. Crystalline index is significantly affected by MWNTs content and increases by increasing of the MWNTs content. In this context, presumably, the MWNTs act as cores for growing the crystals. In contrast, spinning rate had marginal effect on crystallization of nanocomposite fibers.

Raman spectra of neat RSF and RSF/MWNTs were obtained, using a 632.8 nm excitation wavelength (Figure 9). Raman spectra of bulk MWNTs mostly dominated by characteristic peaks of nanotubes. Four prominent sets of peaks were observed in the low- (~200 cm\(^{-1}\), RBM [radial breathing mode]), moderate frequency (1600 cm\(^{-1}\), G band, and 1300 cm\(^{-1}\), D band) and high-frequency (2600 cm\(^{-1}\), D* band) regions. These peaks are typical of MWNTs Raman spectra and their assignments published elsewhere [29].

The RBM peak for neat MWNTs raised around 281 cm\(^{-1}\) and it shifted to higher wave number around 337 cm\(^{-1}\) for RSF/MWNTs. The RBM shifting to higher wave number
can be translated as lower energy for radial vibration. This can be attributed to smaller nanotubes diameter after RSF wrapping [30]. Although the other Raman peaks changes insignificantly for RSF/MWNTs compared to MWNTs, but the intensity decreases for all Raman bands regarding to RSF interaction [31]. The spectroscopic data reveal no evidence for connectivity of nanotube clusters or individuals in biopolymer matrix. In fact, the barrier forms according to amorphous and/or discontinuous conductive path for electrical charge transfer. Therefore, a comprehensive electrical conductivity measurements needs to be carried out to illuminate the electrical charge transfer in accordance to four probe conductivity tests.

**Fig. 9.** Raman spectra of (1) Neat MWNTs (2) RSF/MWNT.

Electrically conducting composites with volume conductivity more than $10^{-10}$ S/cm are evaluated as an important group for many engineering applications [23]. The percolation theory can be applied to explain the electrically conducting behavior of composites consisting of conducting fillers and insulating matrices. Percolation theory predicts that there is a percolation threshold at which a conductive path is formed in the composite causing the material to convert from an insulator to a conductor. The electrical conductivity of the gel and ice formed MWNT cryogels produced from aqueous 9 wt% MWNT dispersed silk fibroin solutions was $1.8 \times 10^{-4}$ S/cm and $1.9 \times 10^{-2}$ S/cm respectively. This suggests that the aligned porous structures benefit from interconnections between the MWNTs [32].

Another report attempts to produce the electrical conductive silk nanofiber mats with and without CNTs. The four-point probe electrical conductivity tests was carried out for silkworm cocoon fibers and its nanofiber with and without CNTs. The samples were considered as films for the simplicity of calculation. The cocoon fibers had the conductivity of 0.016 S/cm whereas that of nanofibers and nanofibers with CNTs was 0.028 and 0.114 S/cm respectively. The cocoon fibers are the least conductive material. The nanofibers have higher conductivity. The addition of only 1% CNT
significantly increases the conductivity of nanofibers [19]. The effect of MWNTs on conductivity of RSF/MWNTs composite fibers was investigated (Figure 10).

![Graph showing conductivity of RSF/MWNTs nanocomposite fibers as a function of MWNTs loading.](image)

**Fig. 10.** The electrical conductivity of RSF/MWNTs nanocomposite fibers as a function of MWNTs loading (■) As spun fiber (●) thermally treated fiber.

In general, the higher the concentration of nanotubes, the higher the electrical conductivity is obtained for the composite fiber. The conductivity gradually increases until the nanotube loading reaches to the percolation threshold. After percolation point, the conductivity shows a plateau profile versus nanotube content [24]. The conductivity data given for as spun and heat treated RSF/MWNT fiber are in agreement with this general phenomenon. The multiple measurements for each sample ensure the certainty of dependence between conductivity and nanotube loading. It can be clearly seen that at higher nanotube content the higher level of error arise according to lower quality of nanotube dispersion. The heat treatment also demonstrates a positive impact on the conductivity (Figure 10b). A one order of magnitude increase in conductivity was observed after thermal treatment. The volume conductivity data was fitted to a power law in terms of weight fraction of MWNTs (Figure 11).
Fig. 11. The acquired percolation threshold based on the experimental data.

Fig. 12. The DSC thermogram for (1) MWNTs (2) RSF (3) RSF/MWNTs (0.2 %w/w) (4) RSF/MWNTs (0.2 %w/w).

The conductivity is linear with \((W - W_c)\) in a logarithmic scale and the relationship is described by the power law equation of \(\delta = R(W - W_c)^t\) where \(\sigma\) is the conductivity of the composite, \(W\) is the weight fraction of the MWNTs in the composite, \(W_c\) is the critical weight fraction, \(R\) and \(t\) are fitting constants [24]. Figure 11 shows an acceptable fit to the conductivity data when the MWNTs loading is larger than a
critical value. The fitting parameters as shown in Figure 11 has been obtained about 0.12 S/cm, 2.8 wt.% and 0.1447 respectively for R, Wc and t.

Figure 12 compares the thermal transition recorded by differential scanning calorimetry (DSC) from room temperature to 450 °C for RSF/MWNTs composite fibers with different MWNTs content and its neat components.

The residual humidity removes between 50-100 °C and accordingly an exothermic peak appears for all samples including neat MWNTs. The glass transition (T_g) occurs around 120 °C which is less than reported T_g in previously published work. It can be attributed to water content in fibroin polymer structure like a plasticizer. Another endothermic peak observed around 210°C for samples containing RSF, which can be associated to formation of β-sheet structure. This temperature also can be distinguished as a starting point for degradation process once a broad exothermic peak reveals in vicinity of 250 °C. The exothermic and endothermic peaks substantially disappears while MWNTs loading increases for RSF/MWNTs composite fiber. Presumably, it can be attributed to formation of the more crystallite with higher thermal stability. The higher thermal degradation temperature occurs in composite fiber with higher MWNTs loading. For neat fibroin a temperature close to 245 °C was recorded as degradation temperature compared to 270 °C for RSF/MWNTs composite fiber.

Experimental part

Materials

The highly purified MWNTs with average outer diameter of 10-15 nm and average length of 5-15 μm was purchased from Neutrino (LTD) and used without further treatment. The Bombyx mori cocoons wastes were also provided by Abrisham Shomal company (LTD). The formic acid (98 %w/w), methanol (99.9% w/w), sodium carbonate (100% w/w), ethanol (99.9% w/w) and anionic detergent was supplied by Merck. and used without further purification. Also, the deionized water was provided by local suppliers.

Extraction of regenerated silk fibroin

The silk fibroin (RSF) is extracted from Bombyx mori cocoons through degumming process using a marseillus soap (0.5% w/w) and sodium carbonate (0.3% w/w) solution at 100 °C for 1 hour. The degummed sericin free fibroin is rinsed thoroughly using warm deionized water for several times. Then, the RSF filaments were dried at 40°C for 24 hours. The RSF solution can be prepared using variety of procedures. However, a tertiary solvent system of CaCl_2/H_2O/EtOH (mole ratio of 1/8/2) has been preferred for 30 min at 85 °C to provide RSF spinning solution [2]. The tertiary solvent system is prepared by addition of EtOH solvent to calcium chloride aqueous solution. The chopped RSF soaked in the tertiary solvent for half an hour to have easier dissolution process. The whole mixture of RSF in tertiary solvent heated to 80 °C with rigorous stirring. After 2 hours a sticky yellowish RSF solution is produced for further purification using dialysis bag in deionized water to remove calcium chloride and other impurities. The dialysis bag remove impurities with small molecular size (molecular cutoff = 12,000–14,000) during 48-72 hours by refreshment of deionized water each 8 hours. After dialysis process, the separation of calcium chloride is ensured by addition of sodium carbonate to precipitate white calcium carbonate.
Alternatively, sodium hydroxide can be used to sediment calcium hydroxide in case of calcium impurity. Similarly, the chlorine impurity assessment carried out by addition of silver salts to precipitate white color silver chloride. Then, the as prepared fibroin freezes at -28 °C for 48-72 hours to obtain amorphous structure. Finally, the freeze-dried RSF was dried in oven at 50 °C for another 48-72 hours to reach sponge like structure.

**Preparation of spinning dope and as spun fiber**

The RSF sponge was dissolved in 98% formic acid to produce 20% (w/v) RSF/formic acid solution to obtain the RSF dope solution. A Branson Sonifier 250 operated at 30W was used to prepare RSF/MWNTs spinning solution. Various amount of MWNTs, were dispersed in 98% formic acid using sonication for 10 min. The RSF was added to this mixture to prepare a 20 w/w % solution and sonicated for another 10 min. This mixture was then stirred for 12h prior to spinning process (Figure13).

![Diagram showing the protocol used in this study for preparation of RSF/MWCNT dispersion.](image1)

**Fig. 13.** Protocol used in this study for preparation of RSF/MWCNT dispersion.

![Diagram showing the setup of the wet spinning process.](image2)

**Fig. 14.** The setup of the wet spinning process.

A micro syringe pump dispenses the spinning solution using a constant feed rate of 30 μL/min to a rotating coagulation bath at 25 rpm (Figure 14). The rotating speed can be adjusted to control the applied radial tension and consequently the fiber diameter. Methanol, ethanol and acetone were used to investigate the rate of phase
change on the fiber morphology. The semi solid filaments washed twice using double distilled water, dried at room temperature.

**Characterization techniques**

The particle size distribution of CNT dispersions was determined using a dynamic light scattering technique (zeta sizer model ZS (Malvern, UK)) with red laser 632 nm (He/Ne). The system uses non-invasive back scatter (NIBS) technology wherein the optics are not in contact with the sample and back scattered light is detected. The use of NIBS technology reduces multiple scattering effects and consequently size distributions in higher concentrations of sample can be measured. The viscosity of polymer solutions was measured using a Brookfield viscometer (RV-DV II+) using spindle Din 85–87. The viscometer was equipped with software (Rheocalc 2.4) to collect and store data and allowed to be analyzed. Fibre samples were cut after cooling in liquid N2 to obtain circular undamaged cross-sections. Small pieces of fibre were fixed vertically on an aluminum stub using conductive glue. A sputter coater (Dynavac) was used for coating of a thin layer of gold on the cross-section and side wall of the fibres. A fully digital LEO Cambridge/Leica Stereoscan 1455 Scanning electron microscope (SEM) with tungsten filament using 10 kV beam energy was used for morphological studies of the composite fibres. The conductivity of five samples of conductive fibers with similar length (1 cm) and diameter (60±10μm) was measured using the four-point probe technique. A homemade four-probe electrical conductivity cell operated at constant humidity and temperature has been employed. The electrodes were circular pins with separation distance of 0.33 cm and fibres were connected to the pins using silver paint obtained from SPI Pty Ltd. Between the two outer electrodes (1 cm distance) a constant dc current was applied using a Potentiostat/Galvanostat. The potential difference generated between the inner electrodes along the current flow direction was recorded by digital multimeter. The conductivity of the solid fibre was then calculated according to the cross-sectional area of the fibre, the dc current applied and the potential drop across the two inner electrodes. The stress–strain data obtained from a universal mechanical testing machine. A 10 mm gauge length of fiber sample was stretched at a strain rate of 500 μm/min until the samples were broken at 25 °C. Fourier transform infrared spectra (FTIR) were recorded using a Nicolet 560 spectrometer to investigate the conformation and crystallinity of RSF and RSF/MWNTs wet spun fibers in the range of 1000- 2000 cm⁻¹ under an absorbance mode.

**Acknowledgements**

Authors acknowledge the financial and technical support of ‘University of Guilan and Guilan Science and Technology park (GSTP).

**References**


