Abstract

Silk fibers are fibrous protein with unique combination of strength and toughness. Its biocompatibility makes it an ideal candidate for various biomedical applications. We hypothesized that composites consisting of silk and carbon nanotube (CNT) will have superior mechanical properties. This paper describes the production of protein based scaffolds having required mechanical properties and active binding sites for cell attachment and proliferation. To achieve this goal we co-electrospun silk-CNT fibers to fabricate nanofibrous scaffolds for tissue engineering applications. The scaffolds were characterized using Environmental Scanning Electron Microscopy (ESEM), Fourier Transform Infrared (FTIR) spectroscopy, Raman spectroscopy and Wide angle X-Ray Diffraction (WAXD) study. The ideal spinning conditions for generating continuous uniform fibers having diameter of 100 nm or less were determined. Mechanical properties of scaffolds were measured by Kawabata Micro tensile tester and cell-matrix interaction study was carried out using Human chondrosarcoma cells (ATCC HTB94).

1 Introduction

Biocompatible and biodegradable synthetic polymeric materials have been used by researchers to develop biological scaffolds[1-9]. However, many of these scaffolding materials have insufficient mechanical integrity and often induce an inflammatory response[10-13]. The superior mechanical properties of silk fibers and possibility of enhanced biocompatibility, makes it an ideal candidate for scaffolds fabrication[14-17].

The scaffolds for the Tissue Engineering application should mimic the natural extracellular matrix and should be fibrous as well as nano scale with fiber architectures conducive to cell deposition and cell proliferation. Electrospinning is a unique method capable of producing nanoscale fibers from both synthetic as well as natural polymers for biomedical applications[18-21]. The use of electrospinning in the fabrication of various nonwoven materials was reported in 1934 for the first time[22] but it was not until recently that it became a popular method for scaffolds fabrication[19].

We generated nanofibrous composite scaffolds by co-electrospinning method. Silk nanofibers were reinforced by carbon nanotubes (CNT). CNTs are
one atom thick layer of graphite rolled into a cylinder. They are 1 nm in diameter and several microns in length. They are light weight, flexible and with elastic modulus of 1 TPa, tensile strength of 37 GPa and breaking elongation of 6-30 % are the hitherto the toughest material known[23-25]. Since the discovery CNTs by Ijima they have become intensely studied material as the fillers for light weight and high strength composites[26, 27]. We hypothesize that CNTs will improve the mechanical properties of the silk nanofibrous scaffolds.

2 Materials and Methods

2.1 Regenerated Silk and Spinning Dope Preparation

The materials used in this study were purchased from Sigma-Aldrich. Degummed Bombyx mori silk was supplied by Taiwan Textile Research Institute (TTRI). All concentration measurements were done weight /weight (w/w). Degummed Bombyx mori silk fibers were dissolved in 50% aqueous calcium chloride (CaCl2) and dialyzed against deionized water. The dialyzed fibroin solution was frozen for 24 hrs at -20 0 C and then lyophilized to obtain regenerated sponge. This regenerated silk fibroin sponge was dissolved in formic acid (98-100%) to carry out electrospinning. For the fabrication of silk-CNT scaffolds purified CNTs processed by high-pressure carbon monoxide (HiPCO) method were dispersed in formic acid by sonication for 2 hrs. The regenerated silk sponge was added to this mixture and again sonicated for one more hour. This mixture was then stirred for one hour prior to spinning. The spinning dope with or without CNTs was placed in a 3-ml syringe (18-G and spinning angle 45°) and electrospun by varying the processing parameters such as concentration, charge density and distance between tip and the collection plate.

2.2 Nanofiber Nanocomposite Characterization

The morphology of the palladium sputtered electrospun fibers was examined and their diameters were determined by Phillips XL-30 ESEM. The average fiber diameter and its distribution were determined based on 100 random measurements. The composition of silk fibers was characterized by Nicolet Magna-IR 560 FT-IR spectrometer. The structure and crystallinity of fibers were determined by Siemens D500 WAXD. Raman Spectra (Renishaw 1000) were obtained using a 780 nm diode laser. Mechanical properties of both random and aligned nanofibers were determined by KES-G1 Kawabata micro tensile tester at the elongation rate of 0.2 mm/sec. For random mat, strips measuring 4 x 0.5cm were glued on a paper frame and then mounted on Kawabata micro tensile machine and average tensile properties from five samples were measured. The time in seconds required to break the sample was noted and converted to displacement (mm) by dividing it by elongation rate (0.2 mm/sec). The displacement was converted to strain by dividing it by gauge length. The load on the strips was computed as gram force. The specific stress in gms/Tex was then calculated using the following equation...

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\text{Stress(gm/Tex) = \frac{\text{Force(gm)}}{\text{specimen width(mm)}} \times \text{Areal density(gm/m}^2)\]

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\text{The areal density is simply the weight (gm) of the nonwoven test strip divided by the area (m}^2\) of the strip. The stress in gms/tex was converted to MPa using following equation...

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\text{Stress(MPa) = 9.8 x Stress(gm/Tex) x density(gm/cc)}\]

2.3 Cell-Nanofiber Interaction Study

The cell-matrix interaction study was carried out on silk nanofibers, silk nanofibers with 1% CNT and natural degummed silk fibers which are 10-20 μm in diameter. Briefly, the scaffolds (1cm x 1cm) were immersed into a 90/10 methanol/water solution for 10 minutes at room temperature. They were then dried at ambient conditions. 1000000 Human chondrosarcoma cells (ATCC HTB94) maintained in culture using Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 1% L-glutamine were seeded per scaffold. The cell-scaffolds constructs were removed from the culture media on 3, 7 and 14 days. They were washed twice with 1x phosphate buffered saline (PBS) and then subjected to a gradient of ethanol (20%, 50%, 70%, 90%, 100%), each for 10 minutes. They were refrigerated overnight at 4°C.

The scaffolds were immediately coated with palladium. The morphology of cells on scaffolds was examined by Phillips XL-30 ESEM. Cell proliferation assay was carried out on 3, 7, and 14 days as well. Scaffolds being assayed were incubated for 2.5 hours with serum free media...
supplemented with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution. The scaffolds were then vortexed with 500μl acidic isopropanol (0.04 M HCl in absolute isopropanol). The intensity of 200μL of this solution was measured at 595nm.

3. Results and Discussion

The concentration was found to be the most important parameter influencing the fiber diameter produced in the electrospinning process. Continuous uniform fibers of less than 100 nm were obtained at 12-15% concentration with the spinning distance of 10 cm and voltage of 30 kV. The initial Young’s modulus of the as-spun silk nanofibers as calculated from the slope of the initial part of the stress-strain curve was 337 MPa at a breaking strain of 4.6 %. The 1 % CNT reinforced silk nanofibers had an initial modulus of 1904 MPa and breaking strains of 0.4 %.( Figure 1).

Raman spectroscopy proved that CNTs were in the samples (Figure 2). Nanofibers were treated with 90/10 (v/v) methanol/water to induce crystallization. FTIR spectra showed that fibers had strong absorption bands in the 1640-1690 cm⁻¹ range (amide I), 1520-1570 cm⁻¹ range (amide II), and an amide (III) band at 1260 cm⁻¹. The β-sheet bands of the amide I group were noticeable at 1620, 1632 and 1651 cm⁻¹ respectively (Figure 3).
making contacts with underlying nanofibers (Figure 4).

Figure 4: Cell-matrix interaction on various silk scaffolds.

4. References


