Effect of Silk Fibroin Content on Physical and Mechanical Properties of Electrospun Poly(lactic acid)/Silk Fibroin Nanofibers for Meniscus Tissue Engineering Scaffold

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Abstract. This study aims to produce composite nanofibers from polylactic acid (PLA) and silk fibroin (SF) with mechanical properties similar to that of a native meniscus. SF is a natural protein, well known for its good biocompatibility and biodegradability but it has poor mechanical properties compared to synthetic polymers. SF contains proteins which can promote cell adhesion and cell proliferation required for a tissue engineering scaffold. PLA is a popular material that is widely used in tissue engineering. It provides high mechanical properties but lacks bioactivity and cell affinity. Thus, a PLA/SF composite scaffold can improve these drawbacks. In this study, PLA/SF nanofibers were fabricated using an electrospinning process at various PLA and SF ratios (PLA: SF 100:0, 75:25, 50:50, and 25:75). The SF content in the emulsion dominated the fiber diameter, fiber arrangement and processibility of nanofibrous scaffold. With increasing SF content, the %elongation at break of PLA/SF scaffold increased but Young’s modulus decreased. The wettability of electrospun PLA was increased with an increase in SF content.

Key words: PLA, Silk fibroin, Electrospinning, Mechanical properties, Physical properties

1. Introduction

The menisci are crescent, wedge-shaped structure that play an important role in shock absorption, load transfer and joints stability of the knee [1]. Loss of meniscal tissue changes knee biomechanics and homeostasis with degenerative changes to the articular cartilage and higher chance of osteoarthritis. The effectiveness of meniscus repair depends on defect location in regard to the vascular supply. Tears in
outer and middle meniscus are able to heal because there has blood supply. On the other hand, inner meniscus, which is avascular and lack of cells, has poor healing potential [2]. Severe meniscal injuries are treated by partial or total meniscectomy. This method leads to early degeneration of knee joint [3]. Therefore, tissue engineering scaffold can offer a variable solution for this problem.

Tissue engineering is an interdisciplinary field of regenerative medicine for tissue repair after damage caused by a disease or an accident [4]. Typically, there are three components that are involved in tissue engineering: (1) reparative cells (form a functional matrix); (2) bioactive molecules; and (3) bioscaffolds (for transplantation and support). These three components may be used individually or in combination to generate organs or tissues [5]. By the way, possession of the scaffold determines cell adhesion, migration and proliferation of reengineered tissue. The bioscaffolds therefore requires biocompatibility; non-toxicity and conformity with surrounding host tissue and bioactivity that enhance the bonding between host and scaffold interface [6].

![Figure 1 Chemical structures of a) Poly(lactic acid) and b) Silk fibroin](image)

Poly(lactic acid) (PLA) is a synthetic polyester (Fig.1a) which can be produced from renewable resources such as corn, starch, sugar, or other biomass [7]. Synthesized either by ring opening polymerization or polycondensation, PLA hydrolytically degrades into lactic acid which is a byproduct from metabolic activities making it suitable for medical applications [8]. PLA has been widely used in tissue engineering applications due to its biocompatibility, biodegradability and strong mechanical properties. However, the hydrophobic surface of PLA is a limitation for cell adhesion because cells prefer to attach to hydrophilic surfaces. Thus, the modification of PLA is very important to study [9, 10].

Silk fibroin (SF) is a structural protein of silk fibers obtained from Bombyx mori cocoons containing up to 90% of the amino acids alanine, glycine and serine (Fig.1b) leading to antiparallel β-pleated sheet formation in the fibers [11, 12]. SF possess good biocompatibility, toughness, elasticity and biodegradability which makes it a good biomaterial for tissue engineering and regenerative medicine [13]. PLA/Silk fibroin composite fiber membrane provides a good environment for cell growth and proliferation of cells [10, 14].

There are several processes for fabricating scaffolds. Electrospinning has been used for the fabrication of extracellular matrix (ECM) to mimic fibrous scaffolds for several decades. Electrospun fibrous scaffolds provide nanoscale or microscale fibrous structures with interconnecting pores, resembling natural ECM in tissues, and showing a high potential to promote the formation of tissues. Moreover, several approaches for the formation of three-dimensional fibrous scaffolds arranged in hierarchical structures for tissue engineering have also been presented [15].

![Figure 2 Schematic view of electrospinning process](image)
The aim of this present research is to develop scaffold mimic meniscus fibers using electrospun poly (lactic acid) (PLA)/silk fibroin (SF) and explore their potential use as a meniscus tissue engineering scaffold.

2. Materials and Methods

2.1. Materials

PLA commercial grade (4043D) was purchased from NatureWorks. Chloroform RPE was purchased from Carlo Erba Reagents. Formic acid was obtained from Merk. Bombyx mori cocoons were provided by Queen Sirikit Sericulture Center, Nakhon Ratchasima, Thailand. Sodium carbonate (Na₂CO₃, analytically pure) and calcium chloride (CaCl₂, analytically pure) were purchased from Carlo Erba.

2.2. Methods

2.2.1. Silk fibroin extraction. Bombyx mori cocoons were degummed and extracted to obtain silk fibroin. Silk cocoons were boiled in Na₂CO₃ solution for 30 minutes to remove the sericin and washed with distilled water. The degummed silk fibers were dried overnight, dissolved in CaCl₂ solution at 98±2 °C for 60 minutes and filtered. The filtrate was removed into a cellophane membrane, soaked with distilled water. The distilled water was changed three times a day. The regenerated silk fibroin solution was stored at 4°C in a refrigerator. Finally, the silk fibroin solution was freeze-dried for 24 hours to form silk fibroin powder.

2.2.2. Emulsion electrospinning. PLA was dissolved in Chloroform (15% w/v) and SF was dissolved in formic acid (12%/w/v). The solution of PLA and SF were mixed in three different ratios of PLA:SF (75:25, 50:50 and 25:75). The emulsion was created by magnetic stirring for 12 hours. Electrospinning was performed by placing the PLA/SF solution in a 10-ml syringe with 20 needles gauge at a constant flow rate of 2.0 ml/h, which was maintained using a syringe pump. A voltage of 25 kV was applied with a distance of 15 cm between the tip of the needle and the metallic collector, which were covered with non-stick aluminum foil.

2.2.3. Mechanical properties. Mechanical properties were measured by using Instron Universal Testing Machine (Instron 5565, USA) with crosshead speed on 10 mm/min, 1 kN load cell. The electrospun PLA/SF sheets with a 1 cm x 10 cm dimension were subjected to tensile testing. All samples were cut in the flow direction.

2.2.4. Fiber morphology. The morphology of electrospun fibers was observed by field emission scanning electron microscopy (FESEM; Carl Zeiss, Auriga) with gold coating. The diameter of the fibers was measured from the SEM micrographs using image analysis software (Image J) and calculated by selecting 100 fibers randomly.

2.2.5. Surface wettability. Water contact angle was studied to assess the surface wettability properties of the PLA/SF electrospun nanofibers. The initial distilled water volume was used in each measurement after 60 second exposure at ambient temperature. The image of water drops on sample surface were recorded by a USB digital microscope (1600x) and analyzed with Image J software. Three different points were measured for each sample.
3. Results and Discussions

3.1. Morphology

Fig. 3 shows SEM images of electrospun pure PLA and PLA/SF fibers prepared by electrospinning at various ratios (75:25, 50:50 and 25:75). Electrospun pure PLA displays a uniform thick fiber (Fig. 3a, b) having an average fiber diameter of $10.364 \pm 2.601 \, \mu m$ (Table 1). The PLA fiber surface (Fig. 3c) contains small pores randomly distributed on the fiber due to the solvent evaporation. Electrospun PLA75:SF25 sample shows beads on the electrospun sheet (Fig. 3d, e) and big pores on the fiber surface (Fig. 3f). The fiber diameter was determined to be $0.665 \pm 0.218 \, \mu m$. The electrospun PLA50:SF50 sheet (Fig. 3g, h) shows smaller beads and smoother fiber surface (Fig. 3i) having an average fiber diameter of $0.693 \pm 0.295 \, \mu m$. For PLA25:SF75 sample (Fig. 3j, k), there was less fiber on the collector than other compositions due to increased SF, the electrospinning processing difficulty. There are small particles on the fiber surface (Fig. 3l). This ratio is not suitable for processing the PLA/SF scaffold, as it tends to break up into electrospRAY instead.

The mean diameter of PLA/SF (PLA75:SF25 and PLA50:SF50) fiber was less than that of pure PLA, due to the combination effect of different electrical conductivities, surface tension, and viscosity [16]. With the incorporation of SF in the PLA organic phase, a gradient in surface tension and viscosity emerged during the flow of the emulsion. The fibers were drawn with more force resulting in comparatively thinner fibers [6]. With increasing the SF ratio, the electrospun fibers were notably less uniform.

Table 1 Fiber diameter of electrospun PLA and PLA/SF

| Sample       | Fiber diameter (µm) |
|--------------|---------------------|
| PLA          | $10.364 \pm 2.601$  |
| PLA75:SF25   | $0.665 \pm 0.218$   |
| PLA50:SF50   | $0.693 \pm 0.295$   |
| PLA25:SF75   | $6.577 \pm 9.585$   |

3.2. Mechanical Properties

Effect of SF ratio on mechanical properties of electrospun PLA nanofiber sheet measured in flow direction was demonstrated in Fig. 4 and Table 2. PLA scaffold exhibits the highest ultimate tensile strength (1.14±0.09 MPa) and highest Young’s modulus (70.21±4.99 MPa). The PLA75:SF25 and PLA50:SF50 (26.22±10.64% and 17.51±3.89, respectively) scaffolds have higher elongation at break compared to pure PLA scaffold (15.97±1.80%). The PLA75:SF25 scaffold has the highest elongation at break but the lowest ultimate tensile strength. The PLA50:SF50 shows the lowest Young’s modulus.

Apparently, the elongation at break value of the scaffold was improved by adding SF into the electrospinning solution. With the incorporation of SF, the Young’s modulus decreased while the ultimate tensile strength did not show the obvious trend. This may be due to fiber loosening is significantly reducing mechanical properties compared to pure PLA scaffold. Mechanical properties of electrospun fibrous scaffolds are intimately associated with their fiber density and junctions [17].

Although, the PLA was dissolved in chloroform, it would probably have come into contact with formic acid during mixing. PLA is highly susceptible to acid-catalyzed hydrolysis. The PLA molecular weight may be decrease during stir that need to study further.
Figure 3 SEM images of electrospun a-c) PLA; d-f) PLA/SF-75:25; g-i) PLA/SF-50:50 and j-l) PLA/SF 25:75 fiber at different significations (100x, 500x and 10000x).
Figure 4 Tensile stress-strain curves of electrospun PLA and PLA/SF scaffold

Table 2 Mechanical properties of electrospun PLA and PLA/SF scaffold

| Sample       | Young’s Modulus (MPa) | Ultimate tensile strength (MPa) | Elongation at break (%) |
|--------------|-----------------------|--------------------------------|-------------------------|
| PLA          | 70.21±4.99            | 1.14±0.09                      | 15.97±1.80              |
| PLA75:SF25   | 20.51±3.97            | 0.47±0.07                      | 26.22±10.64             |
| PLA50:SF50   | 16.49±8.71            | 1.05±0.43                      | 17.51±3.89              |

3.3. Surface wettability

Surface wettability is an essential characteristic for biomaterials, which influences cell attachment, cell proliferation and cell migration [18]. The wetting behavior of scaffold was investigated by static water contact angle measurement [19].

PLA scaffold was found to be highly hydrophobic with a contact angle of 142.5 ±2° (Fig.5). Contact angle values for PLA/SF scaffolds slightly decreased to 128.08±1° and 112.81±5° indicating improved hydrophilicity for PLA75:SF25 and PLA50:SF50 samples, respectively. With the addition of SF in the emulsion, there was reduction of hydrophobicity due to the presence of natural amino, carboxylic, and other functional groups in the backbone of SF [6].
Figure 5 Water contact angle of PLA and PLA/SF nanofibrous scaffolds. (Inset figures show the variation shapes of contact angle on different scaffolds.)

4. Conclusion
In this study, an electrospun PLA/SF was fabricated by an emulsion electrospinning method. The SF content in the emulsion dominated the fiber diameter, fiber arrangement and processibility of nanofibrous scaffold. With increasing SF content, the elongation at break of PLA/SF scaffold increased but Young’s modulus decreased. The wettability of electrospun PLA was increased with an increase in SF content.

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