

FABRICATION OF SILK FIBROIN AND FLUORESCENT SILK FIBROIN FIBERS VIA ELECTROSPINNING

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Abstract

Fibroin, derived from cocoon, is a natural polymer used widely and increasingly in various fields. In recent years, the development of fluorescent fibroin has gained attention in the field of tissue engineering, because of its biocompatibility, oxygen and water vapor permeability, biodegradability, and ability to be tested non-invasively. In this work, silk fibroin and fluorescent silk fibroin fibers were fabricated via electrospinning, a simple process through which continuous submicron fibers can be produced. Degummed silk was extracted by dissolving in a calcium chloride solution. The fibroin powder was dissolved in formic acid to form 10, 12, and 14 wt.% solutions. Fluorescent fibroin solutions were achieved with the addition of fluoresceine sodium (FS) as a fluorescent dye. The fluorescent solutions were prepared at 10 wt.% fibroin with varying FS concentration at 0.1, 0.3, and 0.5 wt.%, and at 0.5 wt.% FS with varying fibroin concentration at 10, 12, and 14 wt.%. The fibroin solutions were fabricated into fibers via electrospinning using an applied voltage of 20 kV. The surface morphology of silk fibroin fibers and fluorescent fibroin fibers, observed by scanning electron microscope (SEM), showed long continuous fibers. The average fiber diameter, in sub-micron range, was found to increase with fibroin concentration. The fluorescence intensity observed by fluorescence spectrophotometer was found to increase with decreasing fluorescent dye concentration. The functional groups and chemical structure of silk fibroin fibers analyzed by infrared spectroscopy (IR) were not affected by the addition of the fluorescent dye.

Keywords: Electrospinning; Fibers; Fluorescent; Silk Fibroin

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Introduction

Silk is a biomaterial that has long been part of human lives. Beside its lustrous appearance and fibrous structure that leads to textile application, its mechanical integrity, biocompatibility, and biodegradability enable its uses on human bodies. Silk is composed of two main types of protein, fibroin and sericin (Qi *et al.*, 2017). Fibroin exists as fibrous strands of protein with high crystallinity, giving its high strength. Fibroin fibers are adhered together by sericin, the second protein that can be dissolved in polar solvent.

Having mechanical strength, oxygen and water vapor permeability, biocompatibility, and biodegradability, fibroin extracted from silk is suitable for various applications including biomedical application. Many studies have explored possibilities in using fibroin as drug delivery carriers, electronic skin, and scaffolds (Kim *et al.*, 2015a; Farokhi *et al.*, 2019; Wen *et al.*, 2021). Extracted silk fibroin can be fabricated into various forms, such as fiber, film, hydrogel, and sponge, giving it even more flexibility in usage (Kino *et al.*, 2007; Yi *et al.*, 2018). Fibroin scaffold has shown mechanical integrity, surface structure appropriate for cell adhesion, and growth. Yet monitoring of its degradation can be a challenge. A way that allows convenient monitoring of fibroin degradation is via the use of a luminescent agent. Previous researches have shown different ways of embedding fluorescent dyes into fibroin including feeding silk caterpillar with food containing fluorescent dyes and mixing fluorescent dyes directly into fibroin precursor prior to fabrication (Tansil *et al.*, 2012; Kim *et al.*, 2015b; Pang *et al.*, 2019; Liu *et al.*, 2020). One of the most common fluorescent dyes employed biomedically is fluorescence sodium which has been proved to be nontoxic and noncarcinogenic (O'goshi and Serup, 2006).

In this work, we fabricated fluorescent silk fibroin fibers via electrospinning, using silk fibroin extracted from cocoons from the Surin Province of Thailand and fluorescein sodium as a fluorescent dye. Electrospinning was chosen because of its ability to produce submicron fibers that could create a large surface area suitable for cell adhesion. The

fibroin and dye concentrations in precursor solutions were varied. The obtained fibers were characterized for their morphology, chemical functional groups, and fluorescence intensity.

Materials and Methods

In the fabrication of fibroin and fluorescent fibroin fibers, silk fibroin was extracted from a silk cocoon and was later dissolved to form a fibroin solution for electrospinning. The detailed procedure is given below.

Materials

Silk cocoon was *Bombyx mori*, obtained from Ruenmaii-Baimon Part., Ltd., Surin Province, Thailand. Fibroin extraction was performed using several chemicals including sodium carbonate (NaCO_3) from Chemipan, Bangkok, Thailand; calcium chloride (CaCl_2) from Kemaus, Australia. Ajisawa reagent was made up of CaCl_2 , H_2O , ethanol at a ratio of 1:8:2. Formic acid (98%) for electrospinning solution was obtained from Fisher Scientific, United Kingdom.

Silk fibroin extraction

To obtain fibroin from silk, silk cocoon was first degummed using sodium carbonate, with ratio of silk cocoon to sodium carbonate of 1 to 100 by weight. The cocoon was then placed in boiling water to get rid of sericin. The degummed silk fibers were dried in an oven at 105°C for 90 minutes. The remaining fibroin was dissolved in Ajisawa reagent at 60°C for 2 hours and consequently centrifuged at 4°C at 9,000 rpm for 30 minutes to get rid of impurities (Ngo and Bechtold, 2018). The purified solution was dialyzed in DI water to remove calcium chloride and centrifuged again. The fibroin solution was oven-dried at 40°C for 72 hours to obtain fibroin solid film that was later crushed into powder.

Electrospinning of fibroin

Silk fibroin solution was prepared by dissolving extracted fibroin powder in formic acid at room temperature using moderate magnetic bar stirring. The solution concentration was varied at 10, 12, and 14 wt.% fibroin. Calculated amount of fluorescein sodium was added to the fibroin solution to form a fluorescent silk fibroin solution. For all three fibroin concentrations, the dye was added to create fibroin with 0.5 wt.% dye concentration. For the 10 wt.% fibroin solution, the fluorescent dye concentration was also varied to obtain solutions with 0, 0.1, 0.3, and 0.5 wt.% fluorescein sodium.

Table 1. Prepared solution conditions with different silk fibroin and fluorescein sodium concentrations

Silk fibroin (wt.%)	Fluorescein sodium (wt.%)			
10	0	0.1	0.3	0.5
12	0			0.5
14	0			0.5

Table 1 lists the solution conditions with different silk fibroin and fluorescein sodium concentrations prepared in the work.

Each of the prepared solutions was loaded into a plastic syringe and connected to an electrospinning setup. The applied voltage was set at 20 kV and the tip-to-target distance was 15 cm. For each condition, the fibers were collected for about 30 minutes.

Characterizations

The fibroin and fluorescent silk fibroin fibers were characterized as follows:

- Fiber morphology was characterized using a scanning electron microscope (SEM), JEOL, and JSM-6510LV.
- Chemical functional groups of the fibers were characterized using a Fourier transform spectroscope (FTIR), Perkin-Elmer Spectrum Two with Universal ATR.
- Fluorescence properties of the fluorescence fibers were characterized using fluorescence spectrophotometry, Horiba Scientific, and FluoroMax⁺ SpectroFluorometer.

Results and Discussion

Silk fibroin (SF) extracted from silk cocoon was successfully electrospun into fibers. The addition of fluorescence dye, fluorescein sodium (FS), did not impede the electrospinnability. Both silk fibroin and fluorescent silk fibroin fibers were characterized for their morphology, chemical functional groups, and fluorescence ability. Varying concentrations of fibroin and fluorescein sodium affected fiber morphology and fluorescence intensity as described below.

Morphological characterization

Morphological images of silk fibroin and fluorescent silk fibroin fibers were obtained using SEM. Neat silk fibroin fibers are shown in Figure 1.

The fibroin solutions were prepared at different silk fibroin (SF) concentrations, namely 10, 12, and 14 wt.% SF. All of these prepared solutions produced nonwoven continuous fibers at submicron size. With increasing fibroin concentration, the solution became more viscous and the electrospun fibers appeared larger in diameter, at 10 wt.% SF, the solution was quite dilute such that a few beads formed along the fibers.

The fluorescence silk fibroin fibers were prepared from solutions with different fluorescein sodium (FS) concentrations. With a fixed fibroin concentration (10 wt.% SF), the fluorescent dye was varied, at 0, 0.1, 0.3, and 0.5 wt.% FS. The SEM images of these varying FS concentrations are shown in Figure 2 (a) to (d). The nonwoven fibers appeared to contain more beads and fuse together (Figure 2 (c) and (d)) at high FS concentrations. The fluorescence fibroin solutions were also prepared at fixed dye concentration (0.5 wt.% FS) with varying fibroin concentrations at 10, 12, and 14 wt.% SF. The SEM images of the fluorescence fibroin at different fibroin concentrations are given in (Figure 2 (d) to (f)). Higher fibroin concentration led to higher solution viscosity and thus the larger fiber diameter similar to the neat silk fibroin conditions. However, the fluorescence fibroin fibers in Figure 2 (d) to (f) appeared larger in size than the neat fibroin fibers in Figure 2 (a) to (c). The dye addition even at minimal amount affected fiber morphology. The fiber diameter information plotted in Figure 3 demonstrates the difference among various prepared solutions. The fluorescence fibers fabricated from 10, 12, 14 wt.% SF and fixed 0.5 wt.% FS attained larger diameters than the fibroin fibers prepared from 10, 12, and 14 wt.% SF without dye (0 wt.% FS). Fibers prepared from 10 wt.% SF at varying dye concentration yielded a diameter size around 100 nm. At higher dye concentrations, the fibers contained beads, and the fiber diameters, which were measured from the fibrous portion and not the bead portion, were slightly smaller than fibers at lower dye concentrations.

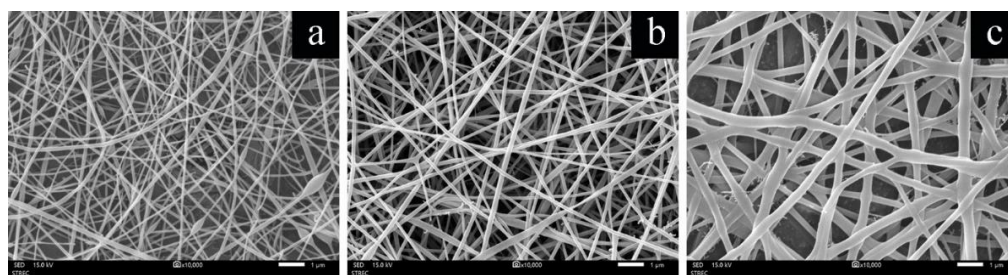


Figure 1. SEM images of neat silk fibroin fibers prepared from solutions with different silk fibroin (SF) concentrations (10,000x): (a) 10 wt.% SF, (b) 12 wt.% SF, and (c) 14 wt.% SF. Scale bar is 1 μm

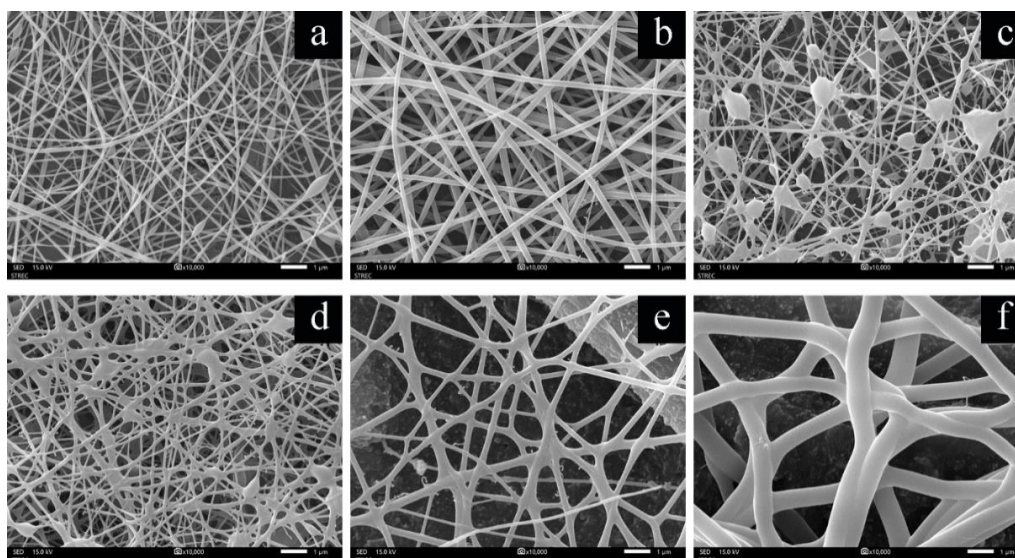


Figure 2. SEM images of fluorescent silk fibroin fibers prepared from solutions with different silk fibroin (SF) and fluorescein sodium (FS) concentrations (10,000x). Top row at 10 wt.% SF (a) 0 wt.% FS, (b) 0.1 wt.% FS, and (c) 0.3 wt.% FS. Bottom row at 0.5 wt.% FS (d) 10 wt.% SF, (e) 12 wt.% SF, and (f) 14 wt.% SF. Scale bar is 1 μm

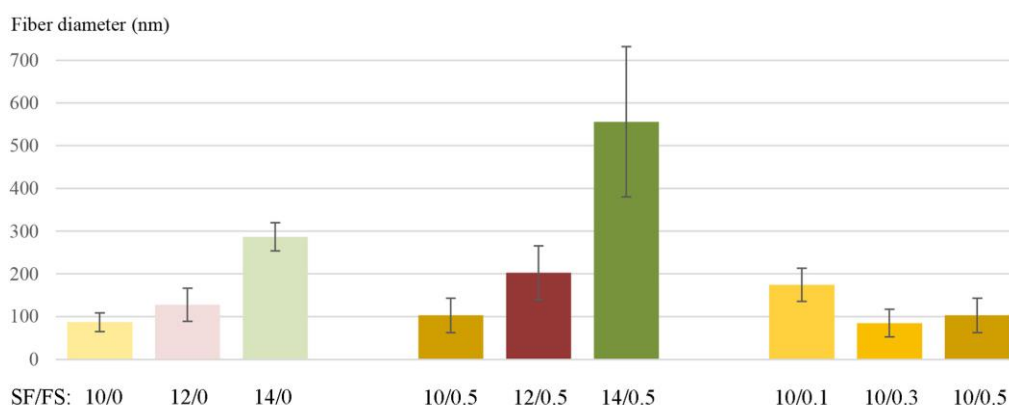


Figure 3. Fiber diameter size of the electrospun fibers observed via SEM, prepared from solutions with different silk fibroin (SF) and fluorescein sodium (FS) concentrations

Chemical functional groups characterization

Fibroin and fluorescent fibroin fibers were examined with FTIR to obtain functional group information. Using infrared wavelength in the range of $4,000\text{--}700\text{ cm}^{-1}$. The spectra are shown in Figure 4. The spectra of both fibroin and fluorescent fibroin fibers show absorption peaks of the fibroin fibers at $3,275\text{ cm}^{-1}$, $1,627\text{ cm}^{-1}$, $1,523\text{ cm}^{-1}$, at $1,237\text{ cm}^{-1}$, corresponding to N-H stretching (Amide A), C=O stretching (Amide I), N-H bending (Amide II), and C-H stretching (Amide III) accordingly. These functional groups verified that both types of fibers were made up of fibroin molecules. The fluorescent fibroin spectrum did not exhibit any other different peaks. This is due to the fact that the dye addition

was negligible such that no dye absorption peak was observed. A similar result was reported by Pang *et al.* (2019).

Fluorescence characterization

The effect of fluorescent dye concentration on fluorescence intensity of the fluorescent fibroin fibers was examined using a fluorescence spectrophotometry with an excitation wavelength of 430 nm and emission spectra in a detectable range of 450–700 nm. The spectra are shown in Figure 5. The fluorescence fibroin fibers exhibited fluorescence spectra in the range of 520–526 nm (green color). It was found that the fluorescence intensity tended to be higher for fibers with lower

fluorescein sodium (FS) solid concentration (concentration of dye within fibers). Dye concentration within fibers is the dye weight over the fiber weight, which consisted of silk fibroin and fluorescein sodium. Among the fibers prepared from 10 wt.% SF, the fibers with the lowest dye concentration (SF10/FS0.1) emitted the highest fluorescence intensity, while the fibers with the lowest dye concentration (SF10/FS0.5) emitted the lowest fluorescence intensity. Among all the fibers prepared from solutions with fixed dye concentration at 0.5 wt.% FS, the SF10/FS0.5 fibers with the highest dye concentration yielded the lowest fluorescence intensity value. The SF12/FS0.5 and SF14/FS0.5 fibers did not follow the trend exactly. Specifically, SF12/FS0.5 contained higher dye concentration, but emitted higher intensity than SF14/FS0.5. Yet the intensities of the three conditions with 0.5wt.% FS were in the same low range. The unexpected low fluorescence intensity of the condition SF14/FS0.5 could be because the dye was unevenly distributed in a highly viscous solution (14 wt.% SF).

Fluorescein sodium concentration within fibroin fibers can be estimated from the concentrations of the prepared solutions. The calculated fluorescein sodium concentrations in the solid fibers are given in Table 2. The reason, why the fluorescence intensity tends to decrease when the dye solid concentration increases, relates to the Fluorescence Quenching Theory. According to the theory, at high dye concentration, the dye molecules aggregate such that the fluorescence effect lessens. The previous work by Pang *et al.* (2019) reported the same trend for the fluorescent silk fibroin solution prepared at 0.5-2.0 wt.% dye concentration. In the current work, we explored lower dye concentration range which exhibited even higher fluorescence intensity.

Conclusions

In this work, silk fibroin (SF) extracted from *B. mori* cocoon from the northeastern province of Thailand was electrospun into fibers at submicron diameter range. Addition of fluorescein sodium dye enabled the fibroin fiber to fluoresce. Silk fibroin solution concentration (10, 12, and 14 wt.%) as well as fluorescein sodium solution concentration (0.1, 0.3, and 0.5 wt.%), affected fiber size and morphology. Higher silk fibroin concentration resulted in fibers with larger diameters, while higher fluorescein sodium concentrations tended to produce beads. With small amount of dye added to the fibers, the detected chemical functional groups within the

Table 2. Calculated weight fractions of fluorescein sodium (FS) in the silk fibroin (SF) fibers

Order of fluorescence intensity	Conditions	Weight fraction of FS within SF fibers
1	SF 10 - FS 0.1	0.009959
2	SF 10 - FS 0.3	0.025830
3	SF 12 - FS 0.5	0.348662
4	SF 14 - FS 0.5	0.299597
5	SF 10 - FS 0.5	0.420509

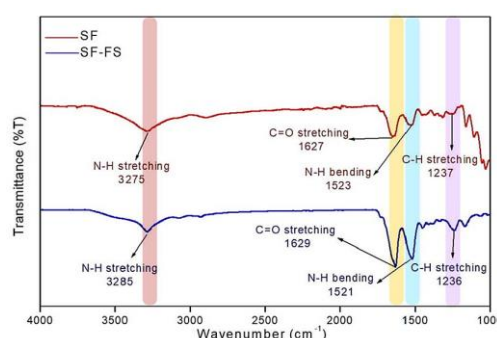


Figure 4. FTIR spectra of silk fibroin fibers (SF) and fluorescent silk fibroin fibers (SF-FS)

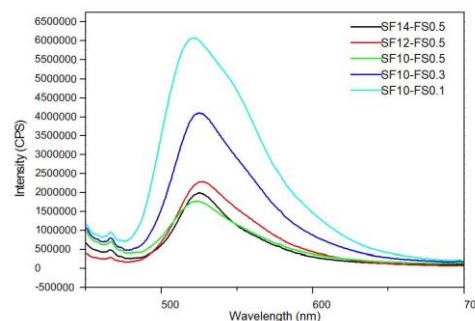


Figure 5. Fluorescence spectra of fluorescent silk fibroin fibers prepared from solutions with different silk fibroin (SF) and fluorescein sodium (FS) weight concentrations

fluorescent fibroin fibers were not different from those within the neat fibroin fibers. Fluorescence intensity of the fluorescent fibroin fibers varied inversely with the fluorescein sodium concentration within the fibers due to the fluorescence quenching effect. Among the prepared conditions, the solution prepared at 10 wt.% fibroin and 0.1 wt.% fluorescein sodium yielded continuous fibers with small diameter around 100 nm with strongest fluorescence intensity. Future work that could be continued includes degradation experiment with which fluorescence properties would be monitored along.

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