



Flexible Bio-Composites Based on Silks and Celluloses

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Biomaterials have attracted worldwide attention due to the concerns regarding health and the environment. Silk, a natural protein produced by several species of insects, has been examined as a potential material for applications in many biotechnological and biomedical fields. However, regenerated silk fibroin has poor ductility and mechanical properties. Therefore, in this study, silk fibroin-cellulose composite films were prepared in an aqueous system to increase the ductility of regenerated silk fibroin. The morphology of the silk fibroin-cellulose composite film was observed by field emission scanning electron microscopy. The structure of the silk fibroin-cellulose composite films was examined by Fourier transform-infrared spectroscopy. The flexibility was analyzed using a bending test.

Keywords: Silk Fibroin, Cellulose, Biomaterial, Flexibility.

1. INTRODUCTION

Biomaterials have attracted considerable attention for their applications to the environment and health. However, the technologies for producing biomaterials require further developments to improve the properties for practical applications in medical fields, such as biosensors, tissue engineering scaffolds and contact lenses.^{1–6}

Silk, a natural biopolymer produced by various species of insects, such as *Bombyx mori*, has been investigated as a potential material for applications in biotechnological and biomedical fields owing to its excellent biocompatible properties, good oxygen permeability and unique mechanical properties. For use as a biotechnological material and biomedical applications, it is essential to regenerate silk fibroin into its proper form. However, the crystallinity of silk fibroin decreases during the regeneration process, resulting in a deterioration of the mechanical properties of the regenerated silk compared to natural cocoon fiber.^{7–8} Moreover, in the wet state, hydrogen bonds form preferentially with water molecules,^{9–10} resulting in poor mechanical strength. Therefore, the possibility of producing silk-based biomaterials by blending with other polymers or adding reinforcements is of great interest.^{11–12}

Cellulose is a polydisperse linear polyglucan that forms a hydrogen-bonded supermolecular structure. It is one of the most abundant biopolymers on Earth and a major component of plants. Therefore, a variety of technologies for using cellulose are being developed over a wide range of applications in paper, textiles, food casings

and biomaterials.^{13–16} However, it is difficult to dissolve cellulose in common solvents due to large number of intermolecular hydrogen bonds of the hydroxyl groups.

Various solvent systems have been developed to regenerate cellulose, such as *N*-methylmorpholine-*N*-oxide (NMMO), LiCl/1,3-dimethyl-2-imidazolidinone (DMI), LiCl/*N,N*-dimethylacetamide (DMAc) etc. However, the toxicity of these organic solvents has restricted their applications as bio-relative materials. Recently, a novel cellulose dissolution technique-based aqueous system was developed using a NaOH/urea aqueous solution. This NaOH/urea solvent system could dissolve the cellulose completely within a short time at low temperatures ($-12\text{ }^{\circ}\text{C}$).^{17–18}

In this study, silk fibroin-cellulose composite films with significantly improved transmittance and flexibility were prepared in an aqueous system. The morphology of the fabricated silk fibroin-cellulose composite film was also examined.

2. EXPERIMENTAL DETAILS

2.1. Preparation of Silk Fibroin Film

Cocoons of *B. mori* silkworm silk were kindly supplied by the UI-gine Sericulture Farm, South Korea. The cocoons were boiled for 30 min in an aqueous solution of 0.02 M Na_2CO_3 , and rinsed thoroughly with water to extract the glue-like sericin proteins. The extracted silk fibroin was dissolved in a 9.3 M LiBr solution at 60 $^{\circ}\text{C}$ for 3 h. This solution was dialyzed in water for 48 h. The final concentration of the aqueous silk solution was approximately

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8 wt%. The silk fibroin film was prepared by solvent casting and treated with methanol for 30 min to induce the β -sheet structure and insolubility in an aqueous solution.

2.2. Fabrication of Cellulose Film

The cellulose film was prepared using the procedure reported elsewhere. An aqueous solution containing NaOH/Urea/H₂O at a weight ratio of 7:12:81 was used as a solvent for cellulose. After pre-cooling the solution to $-12\text{ }^{\circ}\text{C}$, 4 wt% commercial microcrystalline cellulose (Cellulose, powder, $\sim 20\text{ }\mu\text{m}$, Aldrich) was added immediately with vigorous stirring. After 5 min, the cellulose solution became transparent. The solution was poured in a glass dish and dried in a hood for 3 h. The dried cellulose film was immersed in a 5 wt% H₂SO₄ solution to allow coagulation, and dried for 2 days at room temperature. The cellulose films were washed with deionized water until they were neutralized and air-dried at room temperature.

2.3. Fabrication of Silk Fibroin-Cellulose Composite Film

0.1 g of the fabricated cellulose film was immersed in 1.25 g of a 8 wt% aqueous silk fibroin solution (silk fibroin/cellulose: 50/50 (g/g)). After 24 h, the silk fibroin-cellulose composite film was dried in a hood for 2 days. The silk fibroin-cellulose film was treated with methanol for 30 min to induce a β -sheet structure and insolubility in aqueous solutions. The thickness of the final film was $140 \pm 9.46\text{ }\mu\text{m}$.

2.4. Characterization

The morphology of the silk fibroin-cellulose composite film was examined by field emission scanning electron microscopy (FE-SEM, S-4300SE, Hitachi, Japan). Fourier transform infrared (FT-IR, Bruker, VERTEX 80V, Germany) spectroscopy was used to examine the chemical structure of the films. The ultraviolet-visible (UV-vis) spectra were obtained on a Hewlett-Packard 8452A spectrophotometer for the transmittance measurements. A bending test was used to measure the flexibility. The initial transmittance of the silk fibroin film and silk fibroin-cellulose composite film was recorded. The measured silk fibroin film and silk fibroin-cellulose composite film were bent in half. After bending 50 times, the transmittance of the prepared films was measured by UV-vis spectroscopy. The measured transmittance was divided by the initial transmittance to obtain the relative transmittance.

3. RESULTS AND DISCUSSION

Figure 1 shows optical photographs of the silk fibroin film, silk fibroin-cellulose film and cellulose film. In Figure 1,

the silk fibroin film was transparent-like glass. On the other hand, the cellulose film had semi-transparency with a white color. The optical transperance of the silk fibroin-cellulose composite film was similar to that of the silk fibroin film.

The light transmittance of the silk fibroin film, cellulose film and silk fibroin-cellulose composite film were measured using a UV-visible spectrometer. Figure 2 shows the transparency of the silk fibroin film, cellulose film and silk fibroin-cellulose film. The silk fibroin-cellulose composite films exhibited much higher transparency than the cellulose film. The transmittance of the silk fibroin film, silk fibroin-cellulose composite film and cellulose film at 550 nm was 87%, 50% and 6%, respectively. Therefore, the addition of silk fibroin to cellulose leads to an improvement in transparency.

Figure 3 shows the morphology of a cross-section of the silk fibroin film, cellulose film, and silk fibroin-cellulose

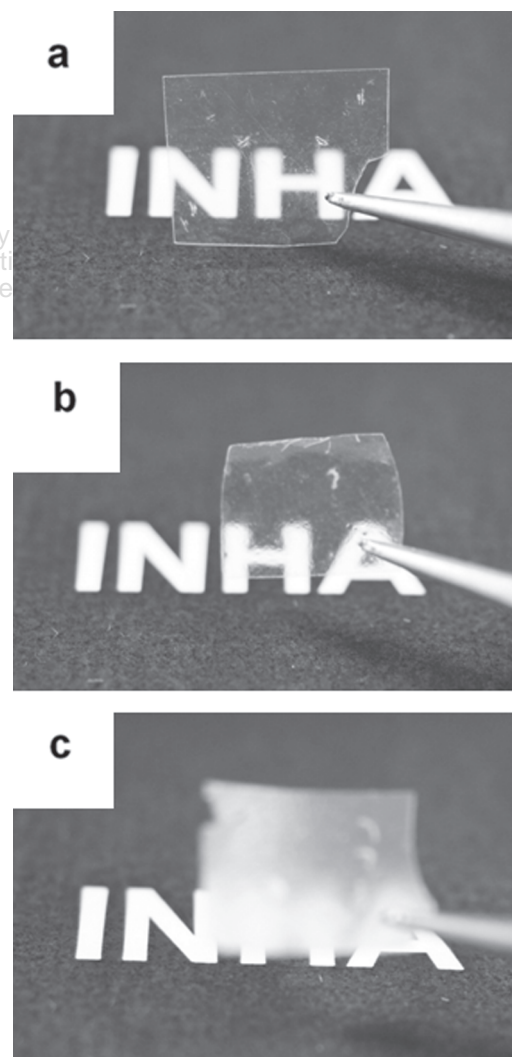


Fig. 1. Optical photographs of (a) silk fibroin film, (b) silk fibroin-cellulose composite film and (c) cellulose composite film.

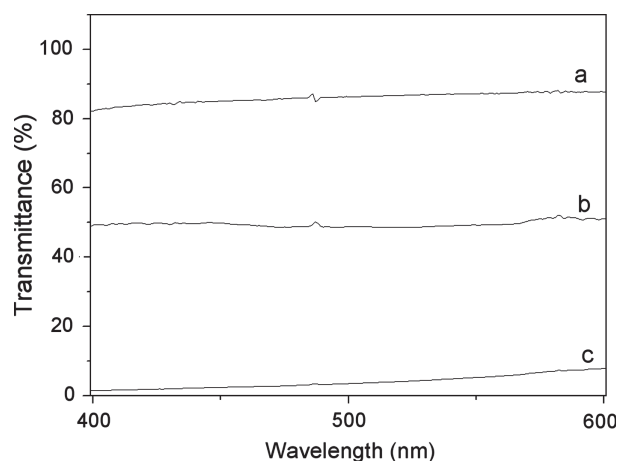


Fig. 2. UV-vis spectra of (a) silk fibroin film, (b) silk fibroin-cellulose composite film and (c) cellulose film.

composite film. The cross-sections of the silk fibroin film showed smooth surface without phase separation. On the other hand, the cross-section of the cellulose film revealed a much rougher surface than the silk fibroin film. The silk fibroin-cellulose composite film showed a smoother surface than the cellulose film. In Figure 3(c), the cellulose film shows a mesh structure with diameters in the hundreds of nanometer range. When the size of the nanofiber in the composite is less than the wavelength of visible light and is composed of a uniform structure, the scattering of light can be ignored despite the difference in the refractive indices of two contents.¹⁹ Therefore, the transparency of the silk fibroin-cellulose composite film is revealed, because a cellulose film with a nano-size mesh structure is impregnated with transparent silk fibroin. However the transmittance of silk fibroin-cellulose composite is lower than that of silk fibroin film due to larger size distribution of the cellulose film diameter.

ATR-FTIR spectroscopy was used to examine the structure of the silk fibroin film, cellulose film and silk fibroin-cellulose composite film (Fig. 4). In Figure 4(a), the silk fibroin film showed β -sheet vibration bands of amide I (1620 cm^{-1} , C=O stretching) and amide II (1514 cm^{-1} , secondary NH bending) due to the methanol treatment, which is a well known method for inducing a β -sheet conformation of silk fibroin.²⁰ The band at 1020 cm^{-1} for the cellulose film in Figure 4(c) was assigned to the C–O–C stretching vibration of cellulose. Figure 4(b) shows the structure of the silk fibroin-cellulose film. The composite film showed a distinctive shift in the amide I band from 1620 to 1654 cm^{-1} . This change clearly shows the formation of the α -helix and random coil structure from the β -sheet structure (silk II) state.

The flexibility of the silk fibroin film and composite film was measured using a bending test (Fig. 5). With increasing bending number, the silk fibroin film exhibited a chalking phenomenon (or whitening)²¹ due to light diffused

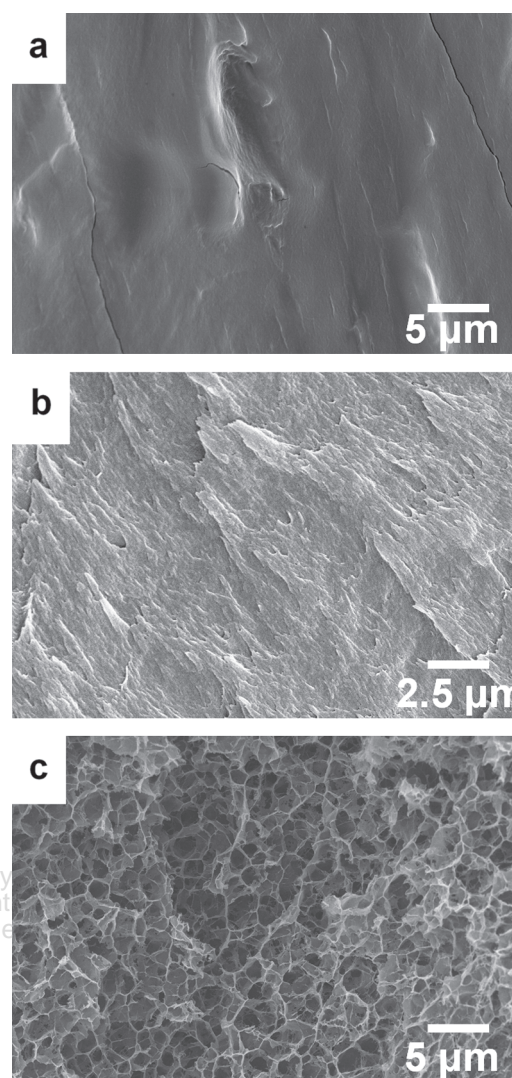


Fig. 3. FE-SEM images of (a) silk fibroin film, (b) silk fibroin-cellulose composite film and (c) cellulose film.

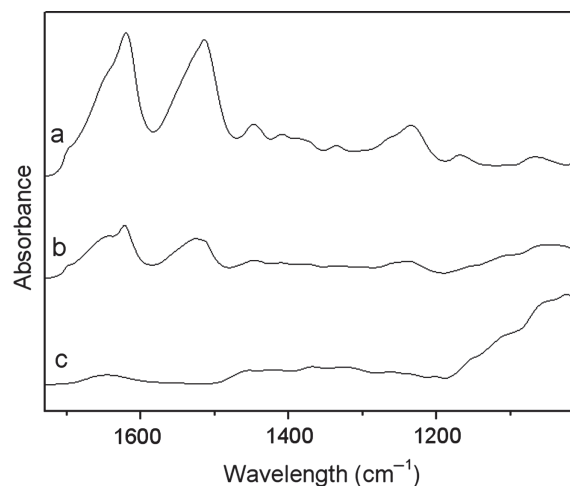


Fig. 4. ATR-FTIR spectra of (a) silk fibroin film, (b) silk fibroin-cellulose composite film, and (c) cellulose film.

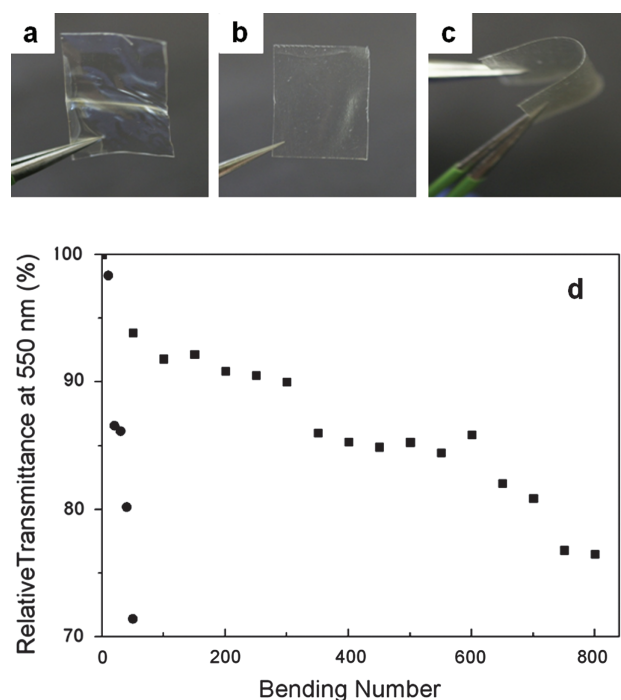


Fig. 5. Optical photographs of (a) silk fibroin film, (b) silk fibroin-cellulose composite film after bending 50 times, (c) bent silk fibroin-cellulose composite film. Relative transmittance at 550 nm (d) of the silk fibroin film (●), silk fibroin-cellulose composite film (■).

reflection based on the diffraction angle of the film according to difference in density. After bending 60 times, the silk fibroin film fractured at the white line. However the silk fibroin-cellulose composite film maintained its shape even after bending 800 times. The bending test results suggest that the flexibility of the silk fibroin-cellulose composite film is increased by the incorporation of cellulose. This was attributed to an increase in the α -helix and random coil structure of the silk fibroin-cellulose composite film, as confirmed by ATR-FTIR spectroscopy.

4. CONCLUSION

Silk fibroin-cellulose composite films were prepared using a simple process. The flexibility of the silk fibroin-cellulose composite was increased due to the increase in α -helix and random coil structure with the incorporation of cellulose. Moreover, the transmittance of the silk fibroin-cellulose composite improved with increasing silk content.

These transparent and flexible silk fibroin-cellulose films are expected to assist in the development of silk based biomaterials for a range of applications.

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References and Notes

- H.-J. Jin, J. Park, V. Karageorgiou, U.-J. Kim, R. Valluzzi, P. Cebe, and D. L. Kaplan, *Adv. Funct. Mater.* 15, 1241 (2005).
- D. L. Kaplan, C. M. Mello, S. Arcidiacono, S. Fossey, K. Senecal, and W. Muller, *Protein-Based Materials*, edited by K. McGrath and D. L. Kaplan, Birkhauser, Boston (1997), p. 103.
- J. M. Deitzel, J. Kleinmeyer, D. Harris, and N. C. Beck Tan, *Polymer* 42, 261 (2001).
- S. Sofia, M. B. McCarthy, G. Gronowicz, and D. L. Kaplan, *J. Biomed. Mater. Res.* 54, 139 (2001).
- Z. Shao and F. Vollrath, *Nature* 418, 741 (2002).
- G. H. Altman, R. Loran, H. H. Lu, J. Moreau, I. Martin, J. C. Richmond, and D. L. Kaplan, *Biomaterials* 23, 4131 (2002).
- H. J. Jin, S. V. Fridrikh, G. C. Rutledge, and D. L. Kaplan, *Biomacromolecules* 3, 1233 (2002).
- Y. Gotoh, M. Tsukada, T. Baba, and N. Minoura, *Polymer* 38, 487 (1997).
- A. J. Poole, J. S. Church, and M. G. Huson, *Biomacromolecules* 10, 1 (2009).
- J. Perez-Rigueiro, C. Viney, J. Llorca, and M. Elices, *Polymer* 41, 8433 (2000).
- E. Marsano, M. Canetti, G. Conio, P. Corsini, and G. Freddi, *J. Appl. Polym. Sci.* 104, 2187 (2007).
- E. Marsano, P. Corsini, M. Canetti, and G. Freddi, *Int. J. Biol. Macromol.* 43, 106 (2008).
- Y. Kim, R. Jung, H.-S. Kim, and H.-J. Jin, *Curr. Appl. Phys.* 9, S69 (2009).
- D. Zabetakis, M. Dinderman, and P. Schoen, *Adv. Mater.* 17, 734 (2005).
- S. H. Yoon, H.-J. Jin, M. C. Kook, and Y. R. Pyun, *Biomacromolecules* 7, 1280 (2006).
- R. Jung, H.-S. Kim, Y. Kim, S.-M. Kwon, H. S. Lee, and H.-J. Jin, *J. Polym. Sci. Pt. B Polym. Phys.* 46, 1235 (2008).
- L. Zhang, D. Ruan, and J. Zhou, *Ind. Eng. Chem. Res.* 40, 5923 (2001).
- A. Lue, L. Zhang, and D. Ruan, *Macromol. Chem. Phys.* 208, 2359 (2007).
- H. Yano, J. Sugiyama, A. N. Nakagaito, M. Nogi, T. Matsuura, M. Hikita, and K. Handa, *Adv. Mater.* 17, 153 (2005).
- Y. Noishiki, Y. Nishiyama, M. Wada, S. Kuga, and J. Magoshi, *J. Appl. Polym. Sci.* 86, 3425 (2002).
- G. Liu, D. Zhu, W. Zhou, S. Liao, J. Cui, K. Wua, and D. Hamilton, *Appl. Surf. Sci.* 256, 2546 (2010).

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