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Solubility and Conformation of Silk Fibroin Membrane

HaeYong Kweon[1,*], Seok-Woo Kang[1], Kwang-Gill Lee[1], KwangYoung Park[1], You Young Jo[1], Pil-Don Kang[1], Gyoo-Byung Sung[1], Heui Sam Lee[1], Joo-Hong Yeo[2], Kyu-Hwan Shin[3], and Chan-Hum Park[4]

[1]*Sericultural & Apicultural Materials Division, National Academy of Agricultural Science, RDA, Suwon 441-100, Republic of Korea*
[2]*Rural Development Administration, Suwon, Republic of Korea*
[3]*Uljin Agricultural Technology Center, Uljin, Republic of Korea*
[4]*Department of Otorhinolaryngology, Hallym University, Chuncheon, Republic of Korea*

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Transparency and insolubility of eardrum patch against exudates are important for otolaryngological surgery. The author prepared silk fibroin (SF) films with various concentrations and temperature and then examined solubility and conformation of SF films. SF films were transparent regardless of the various preparation conditions. Although most SF films are soluble in 1X PBS solution at 37°C for 1 h, the SF film with 3.4% with 60°C was insoluble. Scanning electron microscopy (SEM) showed that the SF films have solid and smooth surface. Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) results showed that the conformation of SF films was influenced by the preparation conditions including SF concentration and casting temperature. In conclusion, SF membrane with transparence and insolubility against exudates could be considered as eardrum patch resources.

Key words: Silk fibroin, Membrane, Solubility, Conformation

Introduction

Silk fibroin (SF) is a typical natural polymer spun by silkworm *Bombyx mori* L. Traditionally SF has been used as a textile fiber and a surgical suture with human beings for centuries. SF has good mechanical and biological properties including low inflammatory reaction (Santin *et al*., 1999), good water vapor and oxygen permeability (Kweon *et al*., 2001; Minoura *et al*., 1990), blood compatibility (Sakabe *et al*., 1989), alkaline phosphatase activation (Kim *et al*., 2010a), and acceleration of collagen formation, and proliferation of cultured human skin fibroblasts (Yeo *et al*., 2000; Yamada *et al*., 2004). Therefore, SF has been attempted in wide varieties of biomedical applications such as matrix for mammalian cell culture and enzyme immobilization (Minoura *et al*., 1995), scaffold for bone substitution (Sofia *et al*., 2001), wound dressing (Kweon *et al*., 2008), and drug delivery carrier (Hanawa *et al*., 1995).

Acute tympanic membrane(TM) perforation is a frequent cause of conductive hearing loss and increases the chance of chronic infection. It is often the result of repeated otitis media or trauma, or it can be a sequel after treatment with ventilating tube (Golz *et al*., 1999). Most acute TM perforations heal spontaneously but a few developed into a chronic stage. Repairing a perforation by office techniques has been successful for a few cases for small traumatic perforations (Kartush 2000). However, healing of bigger perforations is difficult (Ravis *et al*., 1990). It is vital to treat this condition to enable quick growth of a new TM perforation with normal histological characteristics. Surgery is required in case of insufficient healing with medical treatment. However, surgery involves higher costs and more labor, in addition to the surgical risk. Therefore, use of an effective materials for facilitating membrane regeneration, have recently been considered as alternatives to the surgical repair of TM perforation (Amoils *et al*., 1992; Kartush 2000; Kato and Jackler, 1996).

Recently the authors (2010b) reported that transparent silk

*To whom the correspondence addressed
Sericultural & Apicultural Materials Division, National Academy of Agricultural Science, RDA, Suwon 441-100, Korea.
Tel: +82-31-290-8509; Fax: +82-31-290-8516;
E-mail: hykweon@korea.kr

membrane can be used as eardrum patch for regeneration of acute tympanic membrane perforation. The tensile stress of silk fibroin patches (9.7 MPa) was similar to the paper patches (12.5 MPa) but the more flexible (6%) than the paper patches (0.2%). Water contact angle (45.6-52.2) showed that the surface of SF membranes is more familiar to the cell adhesion and growth. Therefore, silk fibroin patches would be more useful than the paper patches. In general, otorhinolaryngologist has treated the perforated eardrum with paper patch. The paper patch is opaque and coarse surface. Therefore, a doctor needs to improve the quality of eardrum patch in the point of the degree of transparency and surface morphology with resistant against exudates. Therefore, the authors prepared SF films to know the feasibility of SF films as eardrum patch.
In this study, the authors prepared SF membrane for eardrum membrane with different preparation conditions and examined its solubility against body fluid model solution. And the morphology and conformation of SF membranes were examined with FT-IR, DSC and SEM.

Materials and Methods

Materials

Silkworm cocoon, harvested in Rural Development Administration (Suwon, Korea), was used for experimental resources. Cocoon was sliced and degummed twice with 0.5% on the weight of cocoon (o.w.c.) Marseilles soap and 0.3% o.w.c. sodium carbonate solution at boiling temperature for 1 h and then washed with distilled water. Degummed cocoon was dissolved in $CaCl_2$:H_2O:ethanol = 1:8:2 in volume. The SF solution was obtained after dialysis against distilled water for 4 d, and then cast on the polystyrene dish to make SF membrane.

Characteristics

To evaluate the solubility of SF films, solubility test was performed. SF films with 5 mm × 5 mm dimension was immersed in the 1X PBS solution at 37°C for 1 hr.

Morphology of SF membrane was obtained through scanning electron microscope (JEOL, JSM 5410LV, Japan) after gold coating.

Fourier transform infrared (FT-IR) absorbance spectra were obtained using FT-IR spectrometer (Spectrum 100, Perkin Elmer, USA) in the spectral region of 2000～650 cm^{-1} at a resolution of 2 cm^{-1} and 32 repeated scans were averaged for each spectrum.

Differential calorimetric properties were measured with a DSC 2910 differential scanning calorimeter (TA instruments Co., USA). The measurements were carried out in the range from 50 to 450°C with a scanning rate of 10°C/min.

Table 1. Solubility of silk fibroin films in the 1X PBS solution at 37°C for 1 hr.

Preparation condition		Solubility*
Concentration (%)	Temperature (°C)	
1.8%	37	○
1.8%	50	○
1.8%	60	○
2.1%	37	○
2.1%	50	○
2.1%	60	○
3.4%	37	○
3.4%	50	○
3.4%	60	×

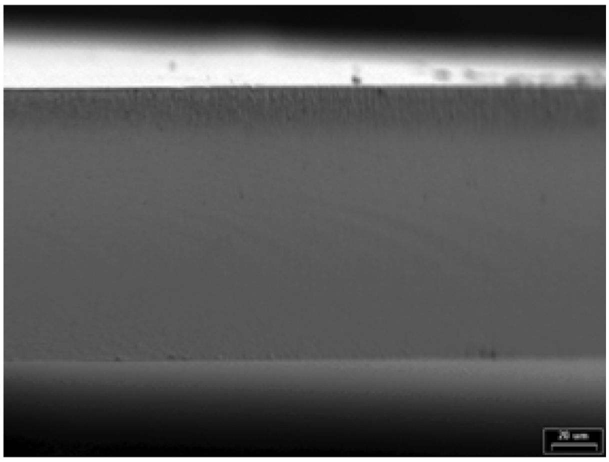
* Solubility is defined as '○' or 'x' whether the silk fibroin film is soluble or not after 1hr. The symbol '○' means silk fibroin film has not been observed with the naked eye.

Results and Discussion

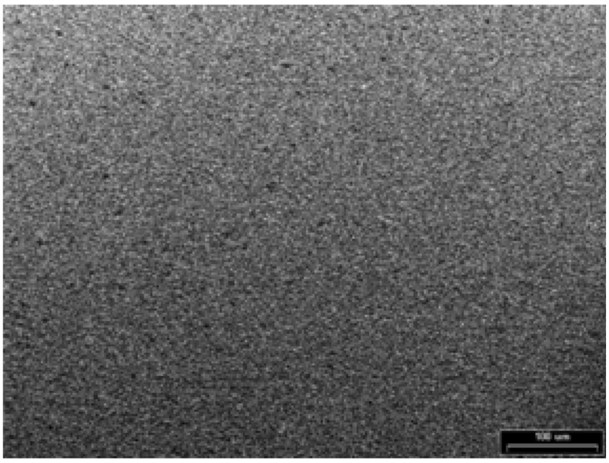
Solubility of eardrum patch membrane is important in the practical viewpoint in otolaryngological surgery. During the treatment on the tympanic membrane perforation, the eardrum patch should not be dissolved in exudation from the eardrum wound. Table 1 showed solubility of SF membrane in model body fluid 1X PBS solution for 1 hr. Solubility is defined as '○' or 'x' whether silk fibroin film is soluble or not after 1hr. The symbol '○' means silk fibroin film has not been observed with the naked eye. Most SF films were soluble in the PBS solution, but SF films cast from high concentration (3.4%) solution with over 60°C were insoluble. When SF films are used for eardrum patch, it is needed to keep the dimension until the tympanic membrane cell covered the defected sites.

The transparency of eardrum patch is useful for the doctor to treat eardrum perforation. The doctor can treat transparent membrane on the defect of perforated eardrum with observation and also observe the curing progress of eardrum through an ear hole. Scanning electron microscopy showed that the typical surface and cross-section morphology of SF membrane. SF membrane is transparent, solid, and smooth surface. In the ear hole, there are many bacteria and foreign bodies, and those are easily seeded and impacted on the irregular surfaces and coarse structure rather than smooth and regular surface. Considering the morphology of SF membrane, it might be good for resistance to the infection and reconstruction of eardrum with easy growth and movement of eardrum cell.

The conformation of SF has been investigated because it is strongly related to the solubility and eardrum surgical performance of SF materials. FT-IR has been used to mea-



(a)



(b)

Fig. 1. The typical scanning electron microgragh of silk fibroin membranes; (a) cross-section and (b) surface.

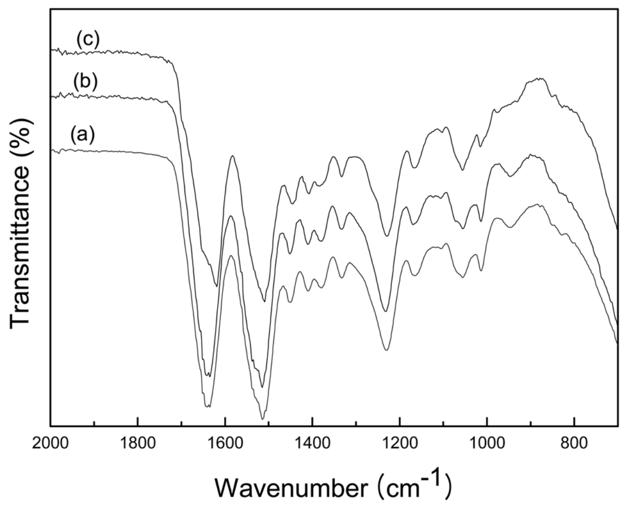


Fig. 2. FT-IR spectra of SF films cast from various concentration of SF solution at 60 ºC ; (a) 1.8% , (b) 2.1%, and (c) 3.4%.

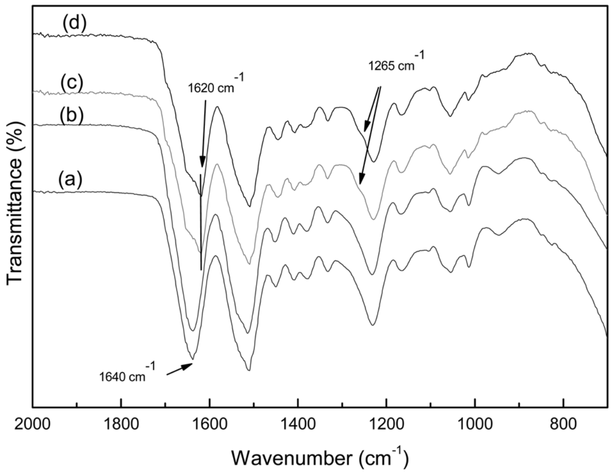


Fig. 3. FT-IR spectra of SF films cast from 3.4% of SF solution with different casting temperature; (a) 37ºC, (b) 50ºC, (c) 60ºC, and (d) 70 ºC.

sure the conformation of SF. The position and intensity of specific absorption bands, the amide bond, are sensitive to the molecular conformation of SF. The absorption bands of SF are assigned as follows; the bands at 1660 (amide I), 1540(amide II), and 1235 cm^{-1} (amide III) were assigned to the random coil structure, the bands at 1630 (amide I), 1530(amide II), and 1265 cm^{-1} (amide III) â-sheet conformation.(Kweon *et al*., 2001, Tsukada *et al*., 1994 Magoahi *et al*., 1985). Fig. 2 showed the FT-IR spectra of the SF films cast from various concentrations of SF solution at 60ºC. The FT-IR spectra (Fig. 2a, 1.8%, and Fig. 2b, 2.1%) of SF membranes showed 1640 (amide I), and 1230 cm^{-1} (amide III), which means that the conformation of the film. On the other hand, SF film cast from 3.4% showed strong absorption peaks at 1650 and 1620 (amide I), 1260 and 1235 cm^{-1} (amide III), which means that conformation of SF was changed β-sheet structure. The effect of casting temperature on the SF film conformation also examined, and the results were shown in Fig. 3. As shown in Fig. 3, the conformation of SF films cast over 60ºC showed β-sheet characteristic absorption bands at around 1620 (amide I) and 1265 cm^{-1} (amide III), which is different with that of SF films cast less than 60ºC. These results confirmed by X-ray diffraction patterns (data not shown). Generally, SF films with random coil conformation are soluble, but SF films with â-sheet structure have a resistance to dissolution.

To confirm the conformation of SF films, differential scanning calorimetric analysis was performed. SF film cast from 1.8% (Fig. 4a) showed the baseline shift at 181.4ºC, strong exothermic peak at 223ºC, and then strong endothermic peak at 282ºC. On the other hand, SF film cast from 3.4% (Fig. 4c) showed strong endothermic

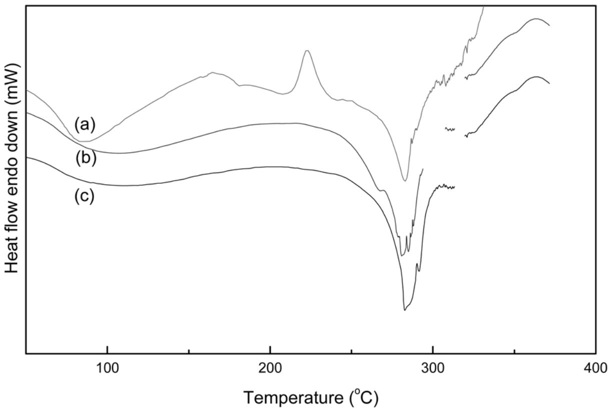


Fig. 4. DSC thermograms of SF films cast from different concentration of SF solution at 60°C; (a) 1.8%, (b) 2.1%, and (c) 3.4%.

peak at 282°C. The exothermic peak at 181.4°C is known as the thermal crystallization behavior of SF protein induced by heat treatment. DSC results about the conformation of SF films are coincident with those of FT-IR.

In conclusion, the author prepared SF films with different preparation conditions including concentration of SF solution and temperature of casting. The morphology of SF films is solid and smooth surface, which is good for resistance to infection during the treatment of eardrum patch. Transparency of eardrum patch and solubility of eardrum patch against exudation are important for practical eardrum surgery. Transparent SF films are helpful for otolaryngologist during the eardrum surgery. The solubility and conformation of SF films could be controlled through preparation conditions of SF films including concentration of SF solution and casting temperature. SF films with transparent, smooth surface and resistance to dissolve against exudation could be promising materials for eardrum patch.

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