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Sericin Film: Influence of Concentration on its Physical Properties

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***Abstract*—**Silk sericin (SS) is a glue-like protein from silkworm cocoon. With its outstanding moisturization and activation collagen synthesis properties, silk protein is applied for wound healing. Since wound dressing in film preparation can facilitate patients' convenience and reduce risk of wound contraction, SS and polyvinyl alcohol (PVA) films were prepared with various concentrations of SS. Their physical properties such as surface density, light transmission, protein dissolution and tensile modulus were investigated. The results presented that 3% SS with 2% PVA is the best ingredient for SS film forming.

***Keywords*—**Sericin, silk protein, film, wound healing.

I. Introduction

SERICIN is a glue-like protein from silkworm cocoon, *Bombyx mori*. It can be separated from insoluble protein called "fibroin" in degumming process. With the properties such as antioxidant and moisturizer, it has been used as a main cosmetic ingredient. Sericin gels increased hydroxyproline in stratum corneum and decreased skin impedance, which shown moisturizing properties [1]. SS protein comprises of three major polypeptides having molecular masses of 150, 250, and 400 kDa, respectively [2]. Tokutake *et al* and Takasu *et al* reported an amino acid composition rich in SS was serine, glycine, and aspartic acid [2,3]. Serine is the major amino acid found in SS protein, however, our previous results showed that methionine play an important role in wound healing process[4] . It is essential in collagen synthesis which is a part of wound healing [5]. Nowadays, there are many researches shown that SS has good wound healing properties. Its component also improves the attachment of cultured human skin fibroblast [6] as well as enhanced effect in promoting corneal wound healing [7]. According to SS cream, it can heal the wound by activating collagen synthesis and wound size reduction without any allergic reaction or inflammation [8,9]. However, using topical preparation for wound healing can generate some disorders such as infection, scars, and itching. Wound contraction, the concentric reduction in size of an open wound [10], is also the one of surgeons' concern. Prevention of contracture is an important goal in the burn wound care. It may cause from insufficient skin surrounding the wound and dehydration [2,7] which may result in contraction, joint inflexibility and tissue stretching. Moreover, treatment of wounds with topical medication is inconvenient because this treatment method is normally processed by physician and cannot prevent scar formation [11]. We hypothesized that SS film will have beneficial over topical preparation and can promote patients' quality of life. Teramoto *et al* showed that the sericin gel film rapidly absorbed water and equilibrated at a content of about 80% without cytotoxicity. With this property, water absorption, is the one of ideal dressing characteristics because moisture can accelerate the rate of epithelialisation and promote healing [12,13]. In order to make SS film, polyvinyl alcohol (PVA), a FDA-approved water-soluble polymer, has been used. This material has excellent film-forming property and good biocompatibility.

The objectives of our study are to formulate SS film at various concentrations with PVA. Their physical properties such as surface density, light transmission, protein dissolution and tensile strength from each formulation have also been investigated.

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II. Materials and Film Preparation

A. Materials

Silk cocoons were kindly supplied from Chul Thai Agro-Industries Co. Ltd., Petchaboon, Thailand. Polyvinyl alcohol, di-sodium hydrogen orthophosphate dodecahydrate were purchased from Ajax Finechem, New South Wales, Australia and sodium dihydrogen orthophosphate dehydrate from Fisher Scientific UK Limited., Leics, United Kingdom.

B. Preparation of SS Solution

Cocoons of silkworm (30 g) were cut into small pieces and put into a 1-litre Duran with distilled water at 1: 15 ratio. It was autoclaved at 121ºC for 60 min then filtered by using white muslin cloth. The SS solution was quite turbid and viscous at room temperature. pH was determined between 6.6-6.8 using Microprocessor pH meter model pH 211 (HANNA instruments, Padova, Italy).

C. Protein Measurement

SS solution was heated to concentrated solution and the amount of protein was measured using BCA™ Protein Assay Kit (Pierce, Tennessee, USA) with microplate reader model UVM-340 (ASYS Hitech GmbH, Austria). The protein concentration has been calculated for film forming.

D. Preparation of SS Films

PVA was dissolved in distilled water at 90ºC for 4 hr and mixed with various concentrations of SS from 1.0-3.0% w/v. The solutions were cast in a petri-dish or plastic tray. Films were left at room temperature overnight then placing in an oven at 60ºC for 24 hr during film-drying process.

III. Analytical Methods

A. Measurement of Surface Density

Each films was weighed on a balance model AB204-S (Mettler Toledo Inc., Ohio, United States), measured width and length by using Vernier Caliper. Weight values were divided by the areas of each sample in order to calculate the surface density (mg/cm^2).

B. Measurement of Light Transmission

Films were cut into a rectangle (approximate 0.9 x 4.0 cm^2) and placed on the internal side a spectroscopy cell. Each sample was recorded the percentage of light transmission values between 400 and 800 nm at 10 nm intervals using a UV/Vis spectrophotometer model Lambda 25 (PerkinElmer Ltd., Bundesverband Solarwirtschaft, Germany).

C. Dissolution Testing

Films were cut into circle shape (diameter 3.8 cm) and attached to aluminum circle sieves in dissolution-testing machine model VK7000 (Varian, Inc., California, USA). Phosphate buffer pH 7.4 containing di-sodium hydrogen orthophosphate and sodium dihydrogen orthophosphate dehydrate was used as a medium. The amount of protein released from SS film to the medium was collected between 0-24 h and analyzed as the method mentioned above.

D. Tensile Modulus Measurement

Modulus values were determined by Universal Setting Machine (Instron 55R4502, Intron Engineering Corp. Canton, MA). All films were cut to 1.5 cm in width and preconditioned at room temperature for 24 h before testing. All experiments were done in triplicate.

E. Statistical Analysis

Data was presented as the mean in percent light transmission and mean ± standard error in others. The statistical significance was determined by One-way ANOVA. A value of $p < 0.05$ was considered to be significant.

IV. Results and Discussion

Physically, all films were clear and quite smooth. Since SS concentrated solution had dark brown color, increasing in SS concentration in film caused more turbidity resulting in the cloudier of samples at higher concentration of SS.

A. Measurement of Surface Density

The surface density values are shown in Fig. 1. Surface density of film containing 3%SS with 2%PVA is significant higher than others ($p < 0.05$). It suggests that increasing concentration of SS raised its surface density values.

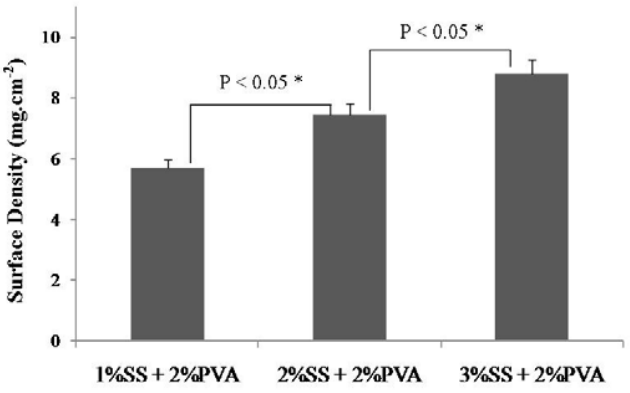

Fig. 1 Surface of SS films

* indicates significant difference when compared to a one step lower concentration ($p < 0.05$)

B. Measurement of Light Transmission

Fig. 2 shows the percent light transmittance of films composed of 1%SS with 2%PVA, 2%SS with 2%PVA and 3%SS with 2% PVA, respectively. Percent light transmission of all samples raised as wavelength increased. Films composed of 3%SS with 2%PVA has the lowest light transmission while films composed of 1%SS with 2%PVA has the most transparency which indicated that higher concentration of SS in films can cause more turbidity.

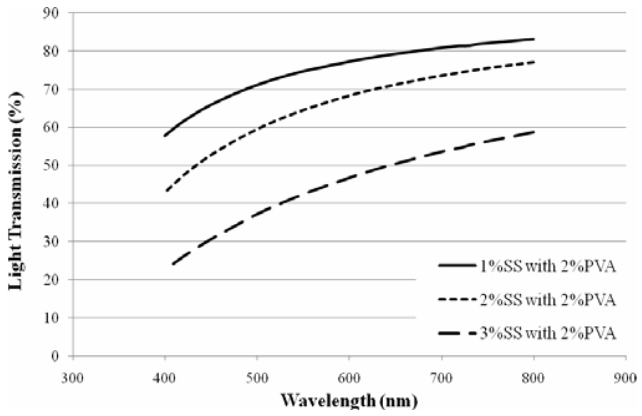

Fig. 2 Percentage of light transmission of SS films

C. Dissolution Testing

Immediately after putting sieves containing SS film into vessels, protein started releasing into buffer solution. Fig. 3 presents protein concentration in collected solution at various time points. At time zero, no SS has been detected but it gradually released with maximum concentration at about 9 h and then gradually decreased which may due to degradation of protein. At initial stage, the slope of protein released from films composed of 1%SS with 2%PVA is higher compared to others which indicated that SS has been released more rapidly.

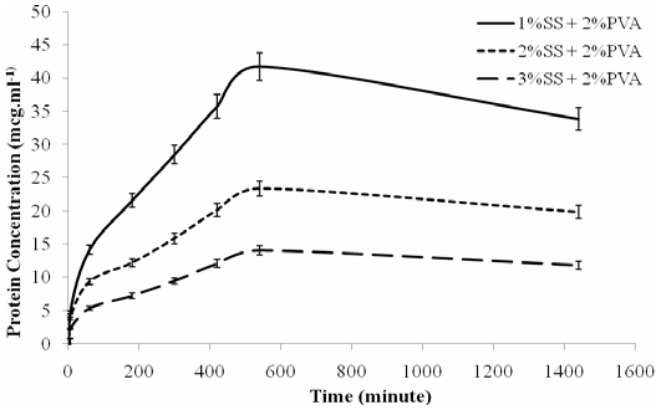

Fig. 3 Dissolution of SS from films at various time points

D. Tensile Modulus Measurement

Fig. 4 represents tensile modulus of SS films. Film composed of 3%SS with 2%PVA had the maximum modulus values compared to others which indicated that this film had the highest elasticity. Although pure SS film was fragile, film composed of SS together with PVA had good mechanical property.

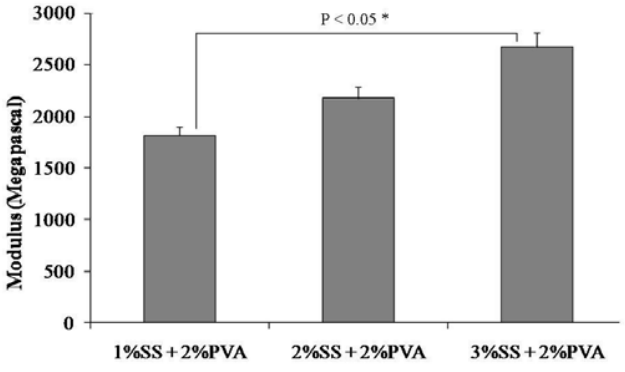

Fig. 4 Tensile modulus of SS films

* indicates significant difference when compared to a two step lower concentration ($p < 0.05$)

Some characteristics of ideal wound dressing are supporting moisture, protecting from microorganism, elasticity, and non-toxicity [14]. According to their tensile modulus, films composed of 3%SS with 2%PVA are suitable for wound dressing advancement. Previous studies showed that modulus of *B. mori* silk (fibroin and SS) and PVA were 5,000-12,000 and 1,700 – 2,100 MPa, respectively [15,16]. Tensile modulus of films composed of SS with PVA may assume between values as mentioned above. Nevertheless, films from all compositions are still bristle and fragile. Adding more plasticizer may improve its properties which need further investigation.

V. Conclusion

SS can be developed as a film for wound dressing. Adding PVA into film formulation can improve its physical property. Due to the elasticity and protein dissolution rate, film composed of 3%SS with 2%PVA is a good candidate for wound dressing development. However, some plasticizer should be added and biological properties of SS film should be further investigated.

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