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Tetracycline-incorporated Silk Fibroin Films

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Silk fibroin films incorporated with tetracycline was prepared and characterized by Fourier-transform infrared spectrometer and differential scanning calorimeter and examined antibacterial effect. The conformation of silk fibroin was changed from random coil to â sheet structure with incorporation of tetracycline. Antibacterial activity of the materials was evaluated against Gram-negative bacteria (Escherichia coli) and Gram-positive bacteria (Staphylococcus epidermis, and Staphylococcus aures) using agar plate method showing clear inhibition zone around tetracycline silk film. It is concluded that tetracycline-incorporated SF films are highly effective against bacteria.

Keywords: Silk fibroin, Tetracycline, Conformation, Antimicrobial, Bacteria

Introduction

Silkworm *Bombyx mori* L. spun cocoon to protect itself from wild environment. The cocoon is composed of fibroin and sericin. Traditionally, silkworm cocoon has been used to make a textile fiber and surgical suture for several thousand years. Recently useful biological properties of silk protein have been reported by researchers; acceleration of collagen formation, and proliferation of cultured human skin fibroblasts (Yeo *et al.,* 2000; Yamada *et al.,* 2004), matrix for mammalian cell culture and enzyme immobilization (Minoura *et al.,* 1995), scaffold for bone substitution (Sofia *et al.,* 2001), and drug delivery carrier (Hanawa *et al.,* 1995), oxygen and water vapor permeability Minoura *et al.*, 1990; Kweon *et al.*, 2001, and blood compatibility (Sakabe *et al.,* 1989).

Tetracycline is a well known antibiotic with activity against both Gram-positive and Gram-negative bacteria. It is widely applied to medicine due to low toxicity (LD_{50} = 6443 mg/kg) and relatively low cost. It exhibits anti-collagenase activity, inhibition of bone resorption, anti-inflammatory action, and an ability to promote attachment of fibroblasts and connective tissue to root surfaces (Bardajee *et al.,* 2011). The biological activities of tetracycline reported are induction of new collagen synthesis (Sasaki *et al.,* 1992), inhibition of phospholipase A2 activity (Pruzanski *et al.,* 1992), inhibition of inducible nitric oxide synthase gene and protein expression (Trachtman *et al.,* 1996), modulation of cytosolic Ca^{2+} response in osteoclasts (Bax *et al.,* 1993), and induction of apoptosis in monocyte and macrophage cell line (Bettany and Wolowacz, 1998).

Therefore, to examine the practical application of silk materials for dental or topical skin treatment, silk fibroin film was cast from dope solution mixed with tetracycline and then examined the structural characteristics and anti-microorganism activity against both Gram-positive and Gram-negative bacteria.

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.

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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 stored in refrigerator to use (Kweon *et al.,* 2011). All chemicals were used as received without any further purification.

Preparation and characterization

Silk fibroin films were prepared through casting method. Tetracycline solution was added into the silk fibroin solution, mixed for 10 minutes and then cast on polystyrene dish to fabricate silk fibroin films incorporated with tetracycline. The concentration of tetracycline was 188 ~ 9460 ppm against the weight of silk fibroin. The films were treated with humid heat at 70°C and 80% RH.

Fourier transform infrared (FT-IR) absorbance spectra were obtained using FT-IR spectrometer (Spectrum 100, Perkin Elmer, USA) in the spectral region of 2000 700 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.

Antibiotic sensitivity is quite significant to develop hygiene membrane for wound area. The effectiveness is base on the size of inhibition zone. Nutrient broth and nutrient agar were supplied by Oxoid. Bacteria used for this study including *Staphylococcus aureus*(ATCC 25923), *Staphylococcus epidermis*(ATCC 12228) obtained from Korean Culture Center of Microorganisms. Primary cultures on nutrient agar slopes and subcultures on nutrient agar plates were stored at 4°C. Antimicroorganism activity against both Gram-positive bacteria, *E. coli, Staphylococcus epidermis, Staphylococcus aures* were examined using agar plate method.

Each inhibition zone was measured one by one with a ruler and then calculated the average value to express the antimicrobial activity of materials.

Results and Discussion

Silk fibroin is a chemically stable material and difficult to change the morphology. Researchers have been used salt solution to dissolve and partially hydrolysis silk fibroin. The silk fibroin solution has been used as the starting materials for various forms of non-textile silk application. Silk fibroin treated with moderated condition salt condition can form film in standard condition. The phase of silk fibroin solution is varied with an additive, for instance, silk fibroin solution is coagulated by addition of alcohol (Nam and Park, 2001). In this experiment, silk fibroin

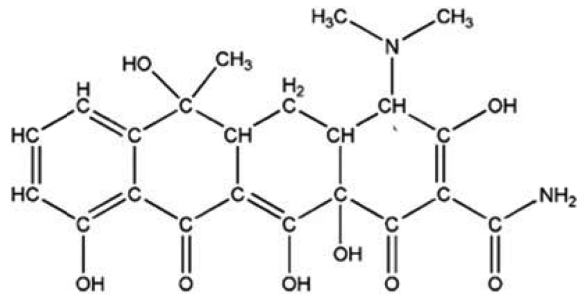

Fig. 1. Chemical structure of tetracycline.

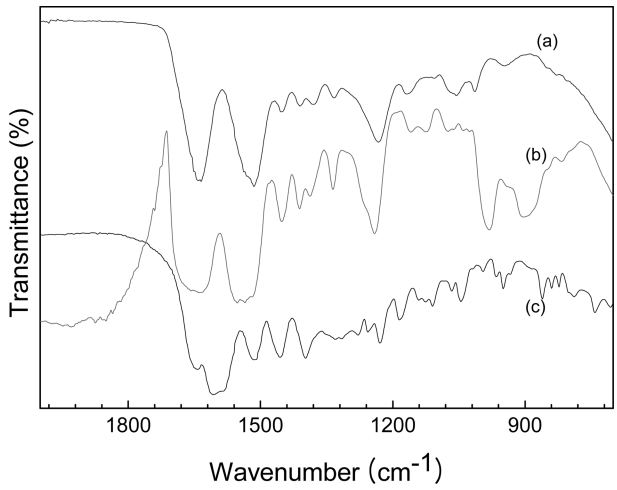

Fig. 2. FTIR spectra of (a) SF, (b) tetracycline-incorporated SF, and (c) tetracycline.

films were successfully fabricated from tetracycline dissolved silk fibroin solution. Tetracycline is widely used as antibiotics in the medical operation. Tetracycline is hydrophilic drug (Fig. 1) with hydroxyl group and amide group.

FT-IR is a powerful technique to examine the conformational changes of silk fibroin. To examine the effect of tetracycline incorporation on the conformation of silk fibroin, FT-IR spectra was measured. FTIR spectra of (a) SF, (b) tetracycline-incorporated SF, and (c) tetracycline were shown in Fig. 2. As shown in Fig. 2(a), SF exhibited a sharp characteristic amide I and II bonds and amide III bonds (1233 cm^{-1}) attributed to the random coil conformation. Tetracycline (Fig. 2(c)) was shown a broad amide I peak and strong peaks around 1515, 1455, 1398, 1256, 1229 cm^{-1}. After incorporation with tetracycline (Fig. 2(b)), amide I and II peaks of SF were broaden and a shoulder peak around 1265 cm^{-1} appeared, which is attributed to the β-structure. Guerra *et al.,* (2005) reported that the characteristic absorption bands of tetracycline are sensitive and shifted with the cooperation to metal ions. Tetracycline, in complex with divalent metal ions, forms very stable complex with peptide (Krafft *et al.,* 1998). From the above

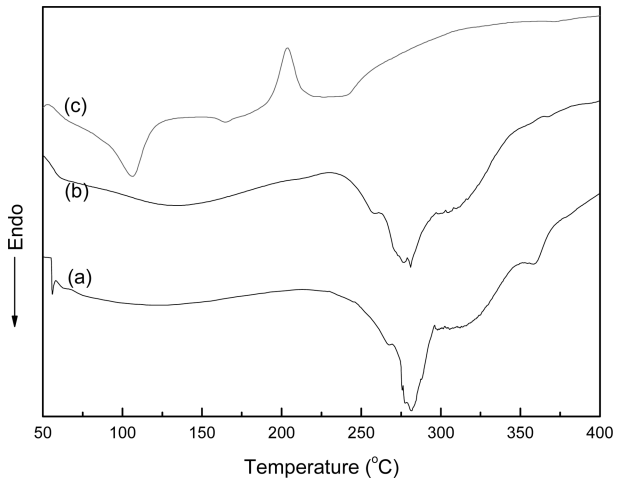

Fig. 3. DSC thermograms of (a) SF (b) tetracycline-incorporated SF, and (c) tetracycline.

Fig. 4. Antibacterial effect of SF loaded with tetracycline to some microorganism after 24hr; (a) *Escherichia coli*, (b) *Staphylococcus epidermis*, (c) *Staphylococcus aureus*.

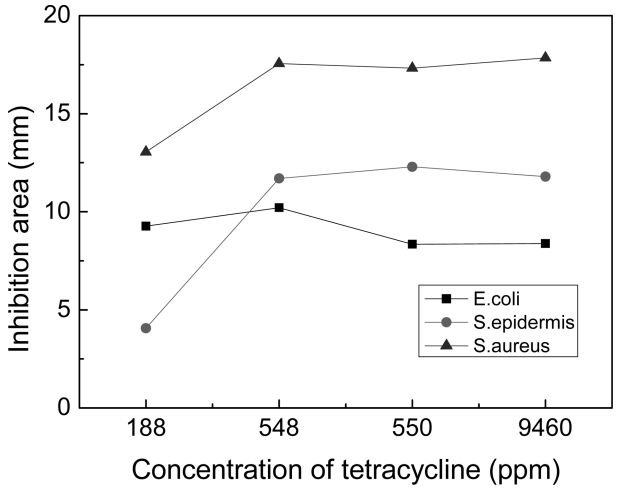

Fig. 5. Effect of tetracycline on the growth of microorganisms after 24 hr.

results showed that tetracycline-incorporated SF films are the mixture of random coil and partial β-structure.

In general, silk fibroin degraded above 280°C due to degradation endothermic reaction. As shown in Fig. 3, tetracycline was shown decomposition peak around 200°C. On the other hand, tetracycline incorporated silk fibroin was shown degradation peak around 280°C. The amount of tetracycline is too small to show the typical thermal properties of tetracycline itself. Therefore, thermal behavior of tetracycline incorporated silk fibroin films looked like that of silk fibroin films.

Antibiotic properties of tetracycline-incorporated silk fibroin film were subjected against *E. coli*, *Staphylococcus epidermis*, and *Staphylococcus aureus*. The SF disc was placed on the agar medium as shown in Fig. 4. The agar plate was kept in an incubator at 37°C for about 24 hr. The bacteria starts grow in the agar medium. If an organism is placed on the agar, it will not grow in the area around the sample disc. Tetracycline silk fibroin exhibited antibacterial effect showing clear inhibition zone due to the release of tetracycline from the films. The inhibition zone of microorganism was increased with the concentration of drug incorporated in silk fibroin films (Fig. 5).

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