설포베타인 키토산의 실크 블렌드 필름의 제조 및 그들의 성질

구자성 · 차재령 · 오세행 · 공명선†

단국대학교 나노바이오의과학과

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Preparation of Sulfobetaine Chitosan, Silk Blended Films, and Their Properties

Ja-Sung Koo, Jae-Ryung Cha, Se-Heang Oh, and Myoung-Seon Gong†

Department of Nanobiomedical Science, Dankook University Graduate School, Chungnam 330-714, Korea
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초록: *Bombyx mori* silk fibroin(SF)과 블렌드 필름을 만들기 위하여 키토산에 1,3-propanesultone을 반응시켜 수용성 sulfobetaine chitosan(SCs)을 제조하였다. 여러 가지 비율의 SF/SCs 블렌드 필름을 *B. mori* SF와 SCs의 수용액을 혼합하여 제조하였다. 수용액으로부터 얻어진 SF/SCs 블렌드 필름의 구조와 형태 변화는 분광학적 및 열적 분석을 통해 규명하였다. SF와 SCs의 혼합 비율에 따른 인공 피부나 화상치료 목적의 비이오재료로서의 물리적 및 기계적 성질에 미치는 영향을 조사하였다. X-선 분석으로 두 생체고분자 사이에 좋은 친화성을 보여주고 있음을 알수 있었으며 기계적 성질도 SCs의 함량이 증가하면 크게 증가하였다. 37 ℃에서 phosphate buffered saline solution 용액 중에서 *in vitro* 분해 실험을 8주 동안 시행한 결과 46.4%가 분해됨을 알 수 있었다. MC3T3-E1 세포에 의한 독성 실험 결과 무독성을 나타내 주었으며, 3일의 배양 후 SF/SCs 필름의 상대 세포 수는 최적화된 tissue culture plastic보다 약간 낮게 나타남을 알 수 있었다.

Abstract: Water-soluble sulfobetaine chitosan (SCs) was prepared for a blending film with *Bombyx mori* silk fibroin (SF) by reacting chitosan with 1,3-propanesultone. A series of SF/SCs blended films were successfully prepared by mixing aqueous solutions of *B. mori* SF and SCs. The SF/SCs blended films were examined through spectroscopic and thermal analysis to determine the morphological changes of SF in the SCs. The effects of the SF/SCs blend ratios on physical and mechanical properties were investigated to discover the feasibility of using these films as biomedical materials such as artificial skin and wound dressing. X-ray analysis showed good compatibility between the two biopolymers. The *in vitro* degradation behavior of the SF/SCs blended films was systematically investigated for up to 8 weeks in phosphate buffered saline solution at 37 ℃ and showed a mass loss of 46.4% after 8 weeks. All films showed no cytotoxicity by MC3T3-E1 assay. After 3 days of culture, the relative cell number on all the SF/SCs films was slightly lower than that of an optimized tissue culture plastic.

Keywords: sulfobetaine chitosan, silk fibroin, blended film, biocompatible polymer.

Introduction

Various natural polymers such as alginic acid, chitosan, collagen, and silk fibroin (SF) have been used as biomaterials for tissue engineering due to their biocompatibility.[1,2] However, they cannot meet the diverse demands for biomaterials as homopolymer alone. The improper mechanical properties and biological activity of homopolymer restrict its application in biomedical materials such as artificial skin and wound dressing.

Many blended films of SF and chitosan have been prepared using the solution blending method to improve the performance of individual natural polymers. However, they cannot be easily processed into blended films with satisfactory stability and mechanical properties using a neutral water solution. Lack of solubility in neutral water and the use of organic acids prohibits adding cell growth factors and limits the use of blended polymers under physiological conditions.[3-5]

SF is a typical fibrous protein that forms silkworm silk filaments and shows unique physical and chemical properties. SF can be used in various forms, such as fiber, film, gel or pow-

†To whom correspondence should be addressed.
 E-mail: msgong@dankook.ac.kr

der, depending on the application.[6-10] Besides its utility as a textile fiber, many researchers have investigated SF as a candidate material for biomedical applications because it has several useful properties including biodegradability, good biocompatibility, good oxygen and water vapor permeability, and produces a minimal inflammatory reaction.[11-13] SF has been used in various fields such as in medical materials for human health, as a food additive, and in cosmetics.

Chitosan and its derivatives are useful in biomedical applications such as wound healing and dressings, drug delivery agents, anti-cholesterolemic agents, blood anti-coagulants, anti-tumor agents, and immunoadjuvants.[14] Chemical modification of chitosan to generate new bifunctional materials is of prime interest because the modification does not change the fundamental skeleton of chitosan, maintains the original physicochemical and biochemical properties, and brings new properties depending on the nature of the groups introduced.[15-17] Several techniques to obtain water-soluble chitosan derivatives have been proposed due to the interesting biological and chemical properties of such polymers. Introducing sulfobetaine onto chitosan through a reaction with 1,3-propanesultone on the amino groups can modify its solubility properties in neutral water.[18,19] Thus the SF/chitosan-like blend films cast from neutral water was possible without dissolving aqueous acid and neutralizing with alkaline solution.

In this study, we determined the structural characteristics and thermal properties of blended films composed of *Bombyx mori* SF and sulfobetain chitosan (SCs). Mixtures of aqueous SF and SCs were prepared in various ratios for the blended films. The structural characteristics, thermal properties, and mechanical properties of blended films were examined through Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), differential scanning calorimetry (DSC), and a tensile tester. We also measured the degradation rate and cytocompatibility test of the SF/SCs blend films, which correlated with the ratio of SF to SCs.

Experimental

Chemicals and Instruments. Chitosan (80% deacetylated, viscosity 200–800 cP, 1% solution in 1% acetic acid Aldrich Chemical Co., St. Louis, MO, USA) and 1,3-propanesultone (Aldrich Chemical) were used as received. *B. mori* silkworm silk was purchased from Boeun Silk Factory in Korea. The swelling ratio was defined as the weight of water absorbed in the swollen film (W_s) divided by the weight of the dried film

(W_d). The swelling ratio was W_s/W_d.

The ^{1}H NMR spectra were recorded on a Varian Gemini-2000 spectrometer at 200 MHz. The FTIR spectra were taken on a Bio-Rad Model MX-3000 spectrometer. DSC data were recorded with a Sinco DSC 131 Evo Setaram DSC instrument. Thermogravimetric analysis (TGA) tests were conducted using a Shimadzu TGA 50 (Shimadzu, Tokyo, Japan) equipment at a heating rate of 10 ^{o}C·min^{-1} under a nitrogen atmosphere. Tensile strength and elongation at the breakpoint of the blended films were measured on an Instron universal testing machine (Model 3344, Instron Engineering Corp., Canton, MA, USA) at a crosshead speed of 10 mm·min^{-1} at room temperature. XRD patterns of the blended films were measured using a Shimadzu XD-D1 X-ray diffractometer and CuKα radiation (λ = 1.54056 Å) at a scanning rate of 2°/sec in a 2θ range from 0 to 30°. The contact angle values were evaluated using PHX 300 software. Surface energy was determined using the Girifalco–Good–Fowkes–Young method and PHX software.

Synthesis of Water-soluble SCs. A mixture of chitosan (3.0 g) dispersed in ethanol (100 mL) was stirred at 90 ^{o}C for 12 h in a round-bottomed flask (250 mL) to swell the chitosan. After the mixture was cooled to room temperature, a solution of 1,3-propanesultone (1.612 g, 13.2 mmol) in ethanol (10 mL) was added dropwise over 30 min. The reaction mixture was maintained at 50 ^{o}C for 5 days with vigorous stirring. After the reaction mixture was filtered, the powdery product was washed with ethanol several times and dried under a vacuum at 50 ^{o}C for 12 h.

FTIR (KBr, cm^{-1}) 3100-3500 (br, O-H and N-H), 1635, 1531 (C=O), 1380, 1150, 1032 (S=O and S-O), 1100 (br, C-O, C-N and C-S). ^{1}H NMR (D$_2$O, ppm) 4.9-4.5 (OH), 3.46 (-NCH$_2$CH$_2$*CH$_2$*S(O$_2$)O-), 2.88 (-N*CH$_2$*CH$_2$CH$_2$S(O$_2$)O-), 1.90 (-NCH$_2$*CH$_2$*CH$_2$S(O$_2$)O-), 2.2 (-COC*H$_3$*). Anal. Calcd: S, 8.99%. Found: S, 8.86%.

Preparation of SF. Silk cocoons of *B. mori* silkworms were degummed using boiling water and Na$_2$CO$_3$ aqueous solution to remove the sericin. The alkaline solution was then drained, and the degummed silk was rinsed several times with water. Finally, the residual sericin was removed completely by rinsing the silk in deionized water and was dried. SF (12%, w/v) was dissolved in a solution of CaCl$_2$/C$_2$H$_5$OH/H$_2$O = 1.0/2.5/8.0 at 80°C for 1 h with continuous stirring. The solution was cooled and then dialyzed against deionized water using a 12000-14000 MW cut-off membrane for 1 week. The water was changed every 24 h. After dialysis, the SF slurry was filtered using a glass-sintered filter with a 300 μm pore size and lyo-

philized to obtain regenerated SF sponges.

Preparation of SF/SCs Blended Films. SF/SCs blended solutions were prepared as follows: A 10% aqueous SF solution (5 mL) and a 5% aqueous SCs solution (10 mL) were mixed in SF:SCs ratios of 5:5 at room temperature. After each 5 mL of the blended solution was taken, air-bubbles were removed under reduced pressure. The clear supernatant solution was used for casting films on an acrylic plate. The casted film was dried at room temperature, washed several times with 30% aqueous ethanol, and dipped in 60% and 100% aqueous ethanol at room temperature for 8 h. The final film was completely dried under reduced pressure. The blend films with SF:SCs = 10:0, 7:3, 3:7, and 0:10 (w/w) were prepared by the similar procedures described above.

SF/SCs = 5:5 blend film: FTIR (film, cm^{-1}) 3282 (O-H and N-H), 2933 (C-H), 1640 (amide I for silk fibroin and *N*-acetyl), 1520 (amide II for silk fibroin and *N*-acetyl), 1365, 1250, 1152 (S-O), 1071, 1031 (C-O and C-N).

In Vitro Degradation Test. SF/SCs blended film (Φ = 12 mm) degradation was quantified by changes in dry weight. The samples were allowed to degrade for 2, 4, and 8 weeks. Dry films were weighed (w_o) and immersed in a conical tube containing 10 mL phosphate buffered solution (approximate pH = 7.3). Degradation was conducted at 37 °C in a water bath. Samples were taken at intervals, rinsed with water, dried in a vacuum oven for 2 days at 50 °C, and weighed (w_t), after which they were discarded. The remaining weight was calculated as: weight remaining(%) = w_o-w_t/w_o×100.

Cell Culture and Proliferation. MC3T3-E1 cells were maintained in standard T75 tissue culture flasks in normal growth medium composed of alpha-modified minimum essential medium (α-MEM) (Invitrogen, Paisley, UK) supplemented with 10% fetal bovine serum (Gibco, Daejeon, South Korea) and 1% penicillin/streptomycin (Gibco). Prior to cell seeding, sections were cut from the synthesized SF/SCs films, placed into 96-well plates, and seeded with 5×10^3 cells suspended in 1 mL normal growth medium and maintained at 37 °C with 5% CO$_2$ for a subsequent time course analysis of cell number. The cells were then incubated for 1 and 5 days, and the cell numbers were measured using the CCK-8 reagent. Absorbance was measured at 450 nm using a spectrophotometer (Bio-Rad, Seoul, South Korea).

Results and Discussion

Preparation of Water-soluble SCs. Water soluble SCs
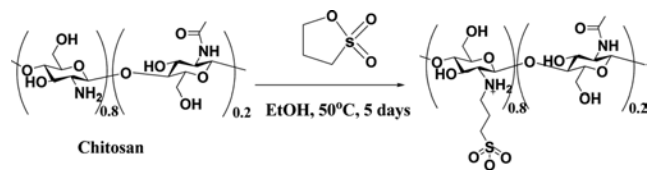
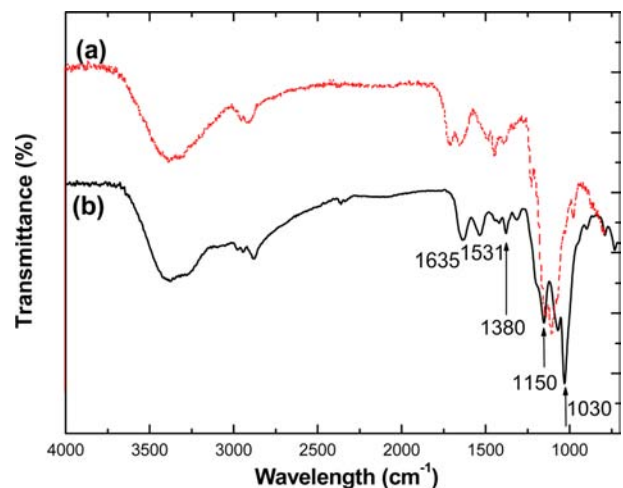**Scheme 1.** Synthesis of watersoluble sulfobetaine chitosan.

Figure 1. FTIR spectra of (a) chitosan; (b) sulfobetaine chitosan (SCs).

was prepared by reacting chitosan with 1,3-propanesultone at 50 °C as shown in Scheme 1. The degree of sulfobetaine in the SCs was calculated by comparing the C and S molar ratio obtained from the elemental analysis. The sulfur content (Calcd: S, 8.99%. Found: S, 8.86%) showed 20% -NHCOCH$_3$, 78.84% -NH$_3$CH$_2$CH$_2$CH$_2$SO$_3^-$ and 1.6% -NH$_2$ in the chitosan backbone. SCs was freely soluble in neutral water and had a viscosity of 16400 cps in a 30% water solution at 20 °C.

Figure 1 shows the FTIR spectrum of chitosan and SCs. A broad peak obtained at 3100-3500 cm^{-1} was due to the O-H and N-H stretching band. The stretching band around 1110 cm^{-1} was due to the C-O, N-O, and C-S groups of the SCs. The characteristic peaks observed at 1152 and 1030 cm^{-1} were attributed to S=O and S-O stretching (Figure 1(b)).

Sulfobetaine formation by the ring-opening reaction was confirmed by ^{1}H NMR of SCs, as evidenced by peaks arising between 2.88 and 3.46 ppm due to methylene protons adjacent to quaternary ammonium and sulfonate, respectively (Figure 2). A multiplet peak at 1.90 ppm was attributed to the methylene protons at the center of sulfobetaine. The sulfobetaine group on the chitosan backbone was calculated from the relative integration of the methylene protons at 2.88 ppm with respect to the methyl protons of -NHCOCH$_3$ at 2.0 ppm, which
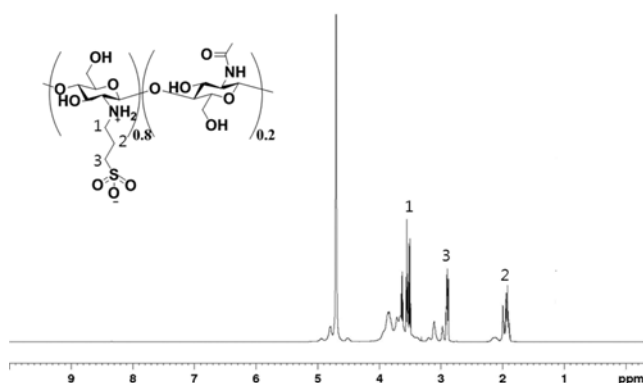
Figure 2. ¹H nuclear magnetic resonance (NMR) spectra of sulfobetaine chitosan (SCs).

matched well with the elemental analysis result.

Preparation of SF/SCs Blend Films. When the aqueous solution of SF was mixed with the viscous SCs solution, the blended SF and SCs solutions produced a viscous hydrogel after standing for 30 min. The SF contents in the cast films were SF/SCs = 10/0, 7/3, 5/5, 3/7, and 0/10. The cast films were transparent and mechanically soft as shown in Figure 3. The SF solution had a slightly negative electric charge (-2.43 mV) and the water-soluble SCs solution had a positive electric charge (+112.0 mV) by the zeta potential. Thus, the SF/SCs blended films possessed an electrical interaction between the SF and SCs.

The pure SCs film was dissolved directly in water, whereas the SF film was swelled and soluble within 24 h in water. The
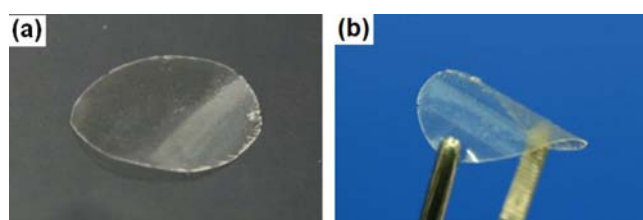
blended SF/SCs-7 film was more swellable (Figure 4(a)) in cold water than that of the SF/SCs-3 (Figure 4(c)) film with swelling ratio of 62. Films containing > 50% SF were resistant to water (Figure 4(c)) and showed swelling ratio of 48. The SF/SCs-3 film was swellable (swelling ratio of 21), but insoluble after 56 days. The swelling ratio in water increased with increasing SCs content. The crosslinked SF/SCs-5 blend film with 2% glyoxal showed a little swelling properties or stability in water.

Characterization of the SF/SCs Blended Films. FTIR spectroscopy was used to follow the conformational changes that occurred during the film process. Figure 5 shows the FTIR spectra of the SF/SCs blended films. The secondary structure of *B. mori* SF consisted of major conformations including random coils (silk I) and β-sheets (silk II). SF showed strong absorption bands at 1640 (amide I), 1520 (amide II), and 1230 cm⁻¹ (amide III), which were attributed to the β-sheet conformation, and some trace of a shoulder at 1528 and 1265 cm⁻¹ were assigned to the random coil conformation. But the content of β-sheet is about 85%. In contrast, the FTIR spectrum of water soluble SCs at 1637 and 1532 cm⁻¹ corresponded to amide C=O stretching. Absorption at 1150 cm⁻¹ was attributed to the symmetric and asymmetric stretching of S=O in -SO₃⁻. The blended films were somewhat different from that of pure SF film in the amide region. The FTIR spectra of the blended films revealed that the amide II band of SF at 1510 cm⁻¹ was shifted to around 1528 cm⁻¹ and that the amide III bands were also shifted to a higher wavenumber

Figure 3. Photographs of (a) SF/SCs-3; (b) SF/SCs-5 films.
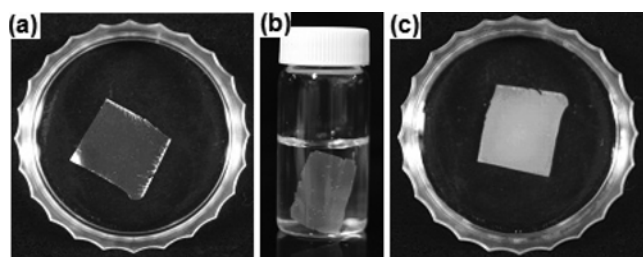
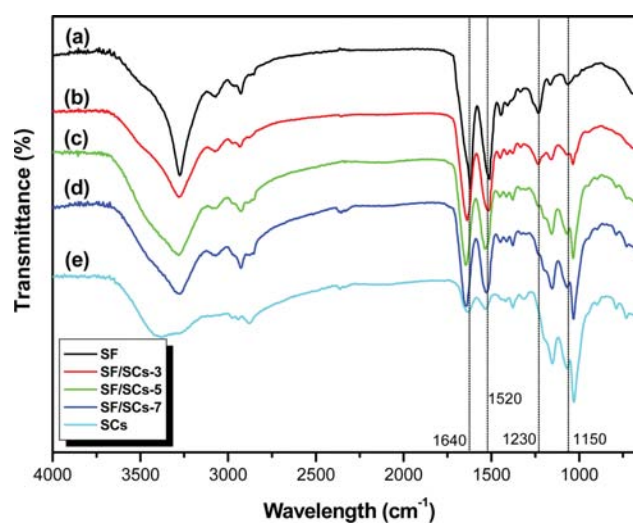**Figure 4.** Photographs of (a) SF/SCs-3; (b) SF/SCs-5; (c) SF/SCs-7 blend films in water.

Figure 5. Fourier transform-infrared (FTIR) spectra of SF/SCs blend films: (a) SF; (b) SF/SCs-3; (c) SF/SCs-5; (d) SF/SCs-7; (e) SCs.

while retaining intensity. Because the characteristic amide (C=O and N-H) groups coexisted in the two polymers, some SCs and SF characteristic bands were combined or shifted as shown in Figure 5. These results demonstrate that the C=O, NH, and zwitterionic groups of SF and the C=O, OH, NH, NH_2^+, and SO_3^- groups of SCs might have participated in a specific intermolecular interaction between the SF/SCs blended films.[20,21]

Thermal Properties of SF/SCs Blend Films. Figure 6 shows the DSC thermograms of SF, SCs, and the SF/SCs blended films. The DSC of pure SCs showed a broad endothermic shift at about 120 °C, which may have been due to the loss of moisture content in the hygroscopic zwitterionic SCs. A sharp exothermic peak at 212 °C followed by a broad exothermic peak may be attributed to decomposition of SCs. Decomposition at >250 °C corresponded to thermal decomposition including dehydration of saccharide rings of SCs and degradation of acetylated and sulfobetaine units. Considering that SCs is composed of a sulfobetaine zwitterion, hydroxyl, and acetyl amine groups, assembling the ions and polar group induced a complex interaction in the SCs chains. However, introducing a sulfobetaine group reduced crystallization.

The pure SF film showed a pattern of amorphous SF with three characteristic thermal transitions. A broad endothermic peak at about 265 °C can be explained through crystallization of SF, which changed the structural transition of SF from a random coil to a β-sheet, followed by the thermal decomposition of SF with an unoriented β-sheet structure.

The SF/SCs blended films displayed similar thermal behavior to the pure SCs film. In the case of the blended film con-
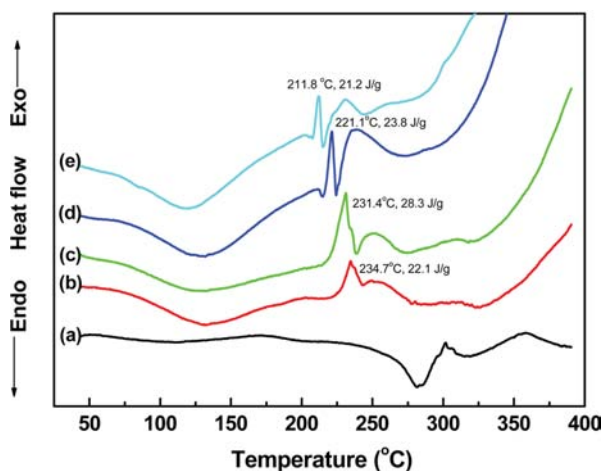
taining SCs, characteristic peaks of pure SCs film attributed to decomposition also appeared. In addition, the exothermic peak showed an upward shift from 218 to 235 °C with increasing SF content.

The TGA curves of SF/SCs blended films are shown in Figure 7. TGA of SCs showed four different weight loss stages. The first stage ranged between 10 and 210 °C. This may have corresponded to the gradual loss of adsorbed and bound water. The second stage of weight loss started at 210 °C and continued up to 250 °C during which there was a 30% weight loss due to dehydration of the SCs saccharide rings. However, the degradation rate changed at about 350 and 460 °C. At these degradation stages, acetylated and sulfobetaine units of SCs occurred and were followed by carbonization of the residual chitosan moiety.

This thermogram was somewhat different from that of the neat chitosan membrane, which degrades in two stages.[22] SCs are thermally unstable. This change might render the material more hydrophilic following sulfonation and destroy the crystalline structure. The structure of the polymer eventually became irregular after sulfonation, which deteriorated the thermal properties. In contrast, *B. mori* SF showed strong thermal decomposition beginning at 240 °C, which was attributed to disintegration of the intermolecular interaction and partial breakage of the molecular structure.

As expected, the SF/SCs blended films showed different weight loss due to decomposition of each component. As SF content increased, the initial thermal decomposition temperature shifted higher and the intensity of the maximum peak varied depending on the blended film composition, indicating
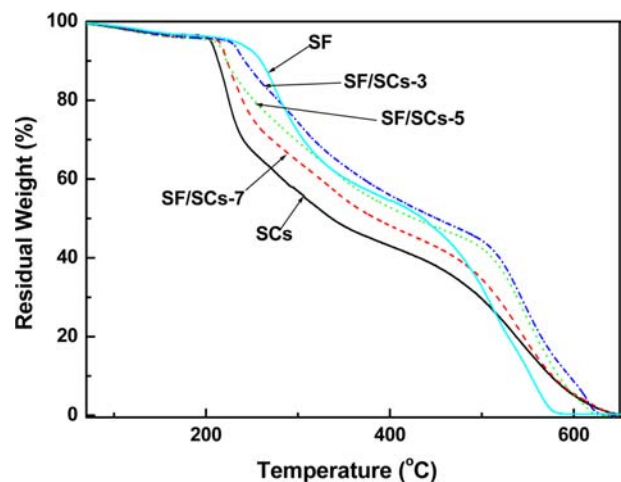

Figure 6. DSC thermograms of SCs, SF and SF/SCs blend films: (a) SF; (b) SF/SCs-3; (c) SF/SCs-5; (d) SF/SCs-7; (e) SCs.

Figure 7. Thermogravimetric analysis (TGA) curves of SCs, SF and SF/SCs blend films.

the formation of an intermolecular interaction between the two components.

XRD Studies. Figure 8 shows the XRD patterns of the SF, SCs, and SF/SCs blended films. The SCs film exhibited typical peaks at $2\theta = 5.6$, 8.2, and 18.9° due to N-sulfobetaine formation, which destroyed the crystalline phase. The diffraction peaks at $2\theta = 8.2$ and 18.9° were assigned to a crystal form of
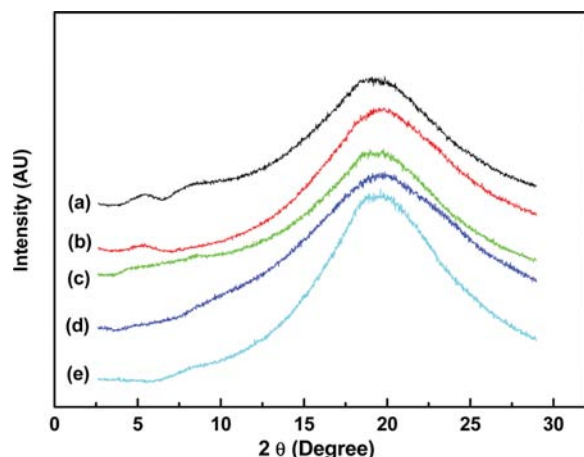

Figure 8. XRD thermogram of SCs, SF and SF/SCs blend films: (a) SCs; (b) SF/SCs-3; (c) SF/SCs-5; (d) SF/SCs-7; (e) SF.

the zwitterionic SCs, which was different from pure chitosan. However, the crystalline peaks at $2\theta = 5.6$ and 8.2° disappeared in the blended films, and a strong peak at $2\theta = 18.9°$ became relatively obtuse and broad, indicating that a large number of ionic interactions and hydrogen bonding occurred between the two polymers through formation of the blend.

Pure *B. mori* SF showed a main diffraction peak at 20° and a minor peak at 7.2°. The weak broad peak corresponding to the β-sheet crystalline structure indicated the low crystallinity of pure SF; thus, pure SF existed mainly in a random coil structure. With an increase in the SCs content of the blends, the intensity of the diffraction peak at 5.6° of SCs decreased, whereas the SF diffraction peak at 20° shifted to a higher angle and overlapped with the main SCs peak at 18.9°. The diffraction patterns of the SF/SCs blended films showed curves in both components that overlapped with each other.

Mechanical Properties of SF/CS Blended Films. The mechanical properties of the films were studied from tensile stress-strain curves as shown in Figure 9 for the blended films. The tensile strength of SF and SCs were 11.8 and 36.3 MPa, respectively. The blended films were somewhat flexible with tensile strengths of 14.8-30.3 MPa (Figure 9(a) and (b)) and
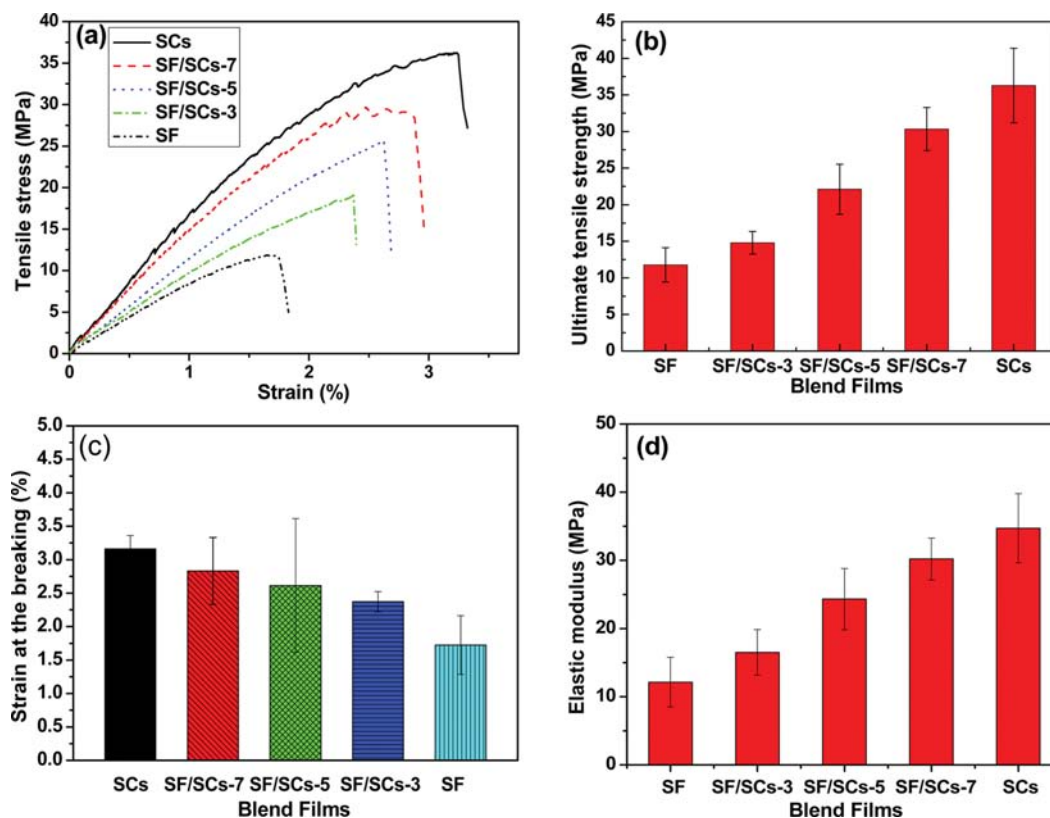

Figure 9. (a) Strain-stress curves; (b) ultimate tensile stress; (c) strain at breaking; (d) Young's modulus for the SCs, SF and SF/SCs blend films.

breaking strains of 1.6-3.3% (Figure 9(c)). The tensile strength of the blended films was higher than that of pure SF and reached a maximum in the SF/SCs-7 blended film. The stress-strain curves of the blended films in Figure 9(a) showed increased tensile stress and initial Young's modulus when the SCs blended ratio was increased (Figure 9(d)), indicating that blending the SCs rendered the films more flexible due the SCs characteristics. The maximum value of the tensile strength with the SF/SCs-7 blend was attributed to structural changes in the SF from a random coil form to a β-sheet crystalline form, resulting in increased modulus and toughness. Figure 9 shows the elastic modulus of the blended films. The SF film exhibited an elastic modulus value because of its brittleness. The maximum increase in the SF/SCs-7 blend was also ascribed to the increase in crystallinity.

Much attention has been paid to surface properties and polymer compositions, because the functional groups on the surface play an important role in the interactions of the materials with cells.[23] Contact angle measurements are a useful technique to estimate surface properties. The measured contact angles and their surface energies are listed in Table 1. SCs (21.7°) had lower contact angle than that of SF (29°) and the water contact angles of the blended films were > 30°. It is generally agreed that hydrophilic surfaces have a contact angle with water in the range 1-30°, whereas hydrophobic surfaces have contact angles > 90°. Therefore, the blended films presented here showed values somewhere between these ranges. As SF content increased, the water contact angle of the SF/SCs blended film increased to 37.7°, suggesting that the films became hydrophobic after blending of the two hydrophilic polymers.

The interaction between two polymers was synergistically promoted by mixing 30 wt% SCs which resulted in a structural change from a random coil form to β-sheet form. We found that proper CS content promoted formation of a β-sheet structure. We suppose that the structural change may be due to the strong hydrogen bonding as well as ionic bonds between SF and SCs.

Table 1. Contact Angles and Surface Energy of SF/SCs Blend Films

Blend films	Water contact angle (°)	Surface energy (mN/m)
SF	29.0±1.2	79.0±0.85
SF/SCs-3	37.7±0.32	72.1±0.28
SF/SCs-5	34.1±0.71	75.1±0.58
SF/SCs-7	30.9±2.6	77.5±1.9
SCs	21.7±3.4	83.5±1.8

In Vitro Degradation Test. Figure 10 shows the degradation ratio of the blended films after different degradation times *in vitro*. The SF film lost about 16.7% of its starting weight, which met the other study's results up to 2 weeks. The SF/SCs films degraded much more quickly during the first 2 weeks. The SF/SCs-3 blend film lost 24.5 wt% of the original weight after 2 weeks. The degradation rate slowed in the next 2 weeks so that 28.6 wt% of the original weight lost after 4 weeks of degradation. The degradation rate became slower in the next 4 weeks. After 8 weeks of degradation, 46.4 wt% (19.28 wt% for silk and chitosan) of the original weight was lost.[24]

The SF/SCs blended films of different compositions were investigated by FTIR spectroscopy to confirm the structural change. The blended films decreased in the broad peak at 3100-3500 cm^{-1} of the assigned OH and NH stretching of the saccharide structure. The characteristic peaks of C=O at 1640 and 1520 cm^{-1} changed to a higher wavelength region at 1655 and 1540 cm^{-1}, indicating that the lower SCs content suppressed crystallization of SF from a β-sheet to a random coil. The other characteristic peaks at 1150 cm^{-1} attributed to -SO$_3$- and S=O were decreased remarkably due to the loss of the SCs portion. Both SF and SCs are regarded as hydrophilic biomaterials in biomedical applications, as they exhibit great hydrophilicity to absorb water. Thus both dissolution of blend film and degradation of hydrolytically sensitive peptide or glycosidic bonds might occur in the degradation test.

Cell Culture and Proliferation. The results of CCK-8 absorbance values for MC3T3-E1 cells adhesion to and proliferation on the SF/SCs blend films are shown in Figure 11. The CCK-8 assay was implemented to measure mouse
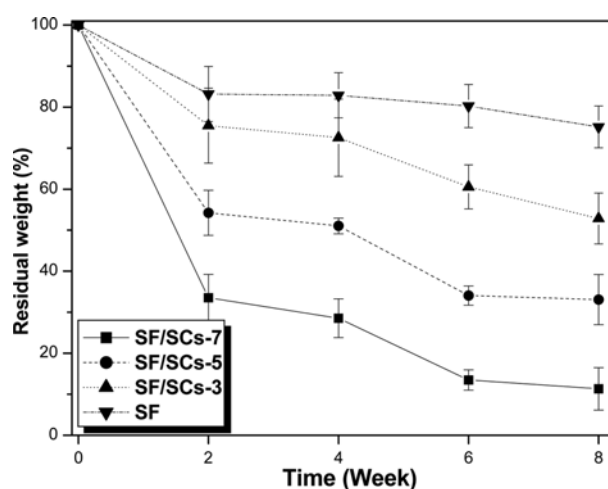

Figure 10. Residual weight of SF and SF/SCs blend films after *in vitro* degradation test in phosphate buffer solution.
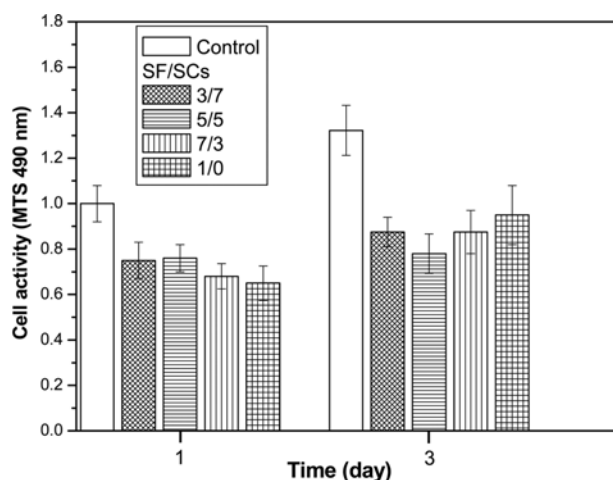
Figure 11. MTS assay of MC3T3-E1 cells cultured on SF/SCs blend films.

MC3T3-E1 cell growth, and as it is specific to viable cells, it reflects not only the ability of the SF/SCs film to promote cell adhesion and proliferation, but also its cytotoxicity. Lower cell viability was observed from 1 to 5 days on the SF/SCs surfaces in comparison with that of the control, because the blend films might be partially dissolved during the cell proliferation. However, the cells began to proliferate and increase in number. Although the cells showed a lower rate of proliferation than that of the control, the SF/SCs films were considered to be cytocompatible.

Conclusions

CS/SCs blended films were prepared, and the FTIR and DSC analyses showed intermolecular interactions between the amide and zwitterionic groups of SF, and the C=O, OH, NH, NH_2^+, and SO_3^- groups of SCs. In addition, the SF and SCs solutions had a negative charge (-2.43 mV) and a positive charge (+112.0 mV), respectively. Thus, the SF/SCs blended films also possessed an electrical interaction between the SF and SCs. The mechanical properties, swelling property, and degradation of the SF/SCs blended films were affected by the proportion of SF and SCs. The SF/SCs blended films had good mechanical properties and water swellability, making them useful as biomaterials. In particular, the blended film containing 30% SCs degraded much more quickly during the first 2 weeks, and the degradation ratio reached 46.4 wt% after 8 weeks. Good hydrophilicity of both SF and SCs produced a large wet/dry weight ratio and the degradation process affected the swelling properties in water. The *in vitro* cell culture

showed that the cells while suppressed slightly compared to the control in general they grew well on these types of blend films up to 3 days. MTS cytotoxicity assay indicated that the silk-sulfobetaine chitosan blends had good biocompatibility.

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