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Research Article

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Preparation and characterization of biodegradable silk sericin/silk fibroin blend microparticles by emulsification-diffusion method

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ABSTRACT

In this study, biodegradable microparticles of silk sericin (SS)/silk fibroin (SF) blends with SS/SF blend ratios of 100/0, 75/25, 50/50, 25/75 and 0/100 (w/w) were fabricated by the water-in-oil emulsification-diffusion method. An aqueous SS/SF blend solution and ethyl acetate were used as the water and oil phases, respectively. The blend microparticles were nearly-spherical in shape with a rough surface. The internal morphology of microparticles was porous structure. The particle sizes slightly decreased as the SF blend ratio increased. FTIR analysis revealed that secondary structure of the SS changed from random coil to β-sheet form by blending with the SF. The dissolution of the blend microparticles strongly depended on the blend ratio.

Keywords: Biodegradable polymers, Silk sericin, Silk fibroin, Microparticles.

INTRODUCTION

Controlled release drug delivery systems have many advantages such as kept drug concentration in plasma at effective level, reduced toxic side-effect and decreased frequencies of drug dosing [1]. Both natural [1-2] and synthetic [3] biodegradable polymers have been widely investigated for use as drug carriers in film, particle, gel and fiber forms for this purpose. The removal of these biodegradable polymer-based devices at the end of therapy is not required.

Silk sericin (SS) and silk fibroin (SF) are hydrophilic natural biodegradable polymers that extracted from silk cocoons. The SS and SF have been widely reported as a biomaterial for use in biomedical [4-6] and pharmaceutical [4,7-9] applications due to its biocompatibility and biodegradability. Both SS and SF have been blended with other polymers to improve their properties [5,7,10-11]. However, SS/SF blend microparticles for drug carrying have scarcely been published. It is well known that the water solubility of the SS and SF is different. This is an important factor for controlling the drug release rate in drug delivery application.

The water-in-oil (W/O) emulsification-diffusion method has been used to prepare many hydrophilic polymer-based microparticles in our research groups such as silk fibroin [12], chitosan [13] and aliginate [14]. The polymer aqueous solution and ethyl acetate were used as water and oil phases, respectively. The main advantages of this method are fast and low-cost.

In this work, the effects of SS/SF blend ratio on morphology, particle size, conformational transition and dissolution behavior of the SS/SF blend microparticles were investigated. The blend microparticles were prepared from SS/SF blend solution in aqueous by the W/O emulsification-diffusion method.

EXPERIMENTAL SECTION

2.1. Materials

A SS aqueous solution was obtained from the *B. mori* silk cocoons. The silk cocoons were boiled in Na_2CO_3 solution (1.0% w/v) at 90 ^oC for 1 h to extract the SS. The SS solution was separated before dialyzing in a cellulose tube (molecular weight cut-off 7,000 Da) for 3 days against distilled water. The distilled water was changed daily. The SS concentration was diluted to 1.0% w/v against distilled water before use.

A SF aqueous solution was prepared from the de-gummed SF fibers. The de-gummed SF fibers were dissolved in the solvent mixture of $CaCl_2$-ethanol-water (1-2-8 mol ratio) by stirring at 90 ^oC for 2 h. The SF solution was then dialyzed in a cellulose tube (molecular weight cut-off 7,000 Da) for 3 days against distilled water. The distilled water was changed daily. The SF solution was diluted to 1.0% w/v against distilled water before use.

Ethyl acetate in analytical grade (Lab Scan) and Span80 (Merck) were used as a continuous oil phase and an oil-soluble emulsifier, respectively.

2.2. Fabrication of SS/SF blend microparticles

The SS/SF blend microparticles were fabricated by the W/O emulsification- diffusion method. The 1% SS and 1% SF aqueous solutions were mixed together in appropriate volume under magnetic stirring for 10 min. The 1.0 mL of a SS/SF blend solution was then slowly added drop-wise to 400 mL of 1% (w/v) Span80 in ethyl acetate under magnetic stirring at 900 rpm. The emulsification-diffusion process took 1 h. The resulted SS/SF blend microparticles were collected and rinsed with fresh ethyl acetate before drying in a vacuum oven at room temperature overnight. The blend microparticles with SS/SF blend ratios of 75/25, 50/50 and 25/75 (w/w) were prepared. The plain microparticles of SS and SF were also prepared by the same method for comparison.

2.3. Characterization of SS/SF blend microparticles

The morphology of microparticls was observed using scanning electron microscopy (SEM, JEOL JSM-6460LV). The microparticles were sputter-coated with gold to enhance the surface conductivity before scanning. The average size of the microparticles was determined from several SEM images by counting a minimum of 100 particles using the smile view software (version 1.02).

Secondary structures of the microparticles were measured using transmission Fourier transform infrared (FTIR) spectroscopy (Perkin-Elmer Spectrum GX) by KBr disc method. The FTIR spectra were determined over the wave number range 500 to 2,000 cm^{-1} with a spectral resolution of 4 cm^{-1} and 32 scans.

A dissolution test of the microparticle samples was studied in a 0.1 mM phosphate buffer solution, pH 7.4 at 37 °C. The sample flask was shaken at 150 rpm for 24 h. The remaining blend microparticles were separated by centrifugation at 5,000 rpm for 30 min. The residue microparticles were then freeze-dried for 24 h before weighing. Equation (1) was used to calculate percentage of dissolution. Each dissolution value was averaged from three experiments.

$$\text{Dissolution (\%)} = \frac{\text{initial weight of microparticles (mg)} - \text{remaining weight of microparticles (mg)}}{\text{initial weight of microparticles (mg)}} \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

3.1. Morphology and size of microparticles

SEM micrographs were used to study the morphology of the microparticles. Fig. 1 shows SEM micrographs of the SS, SF and blend microparticles. It was found that the SS, SF and blend microparticles were a nearly-spherical in shape with a fine dispersibility. The particle surfaces were determined from expanded SEM micrographs as shown in Fig. 2. The surfaces of SS and blend microparticles were rough. Meanwhile, the SF microparticles had smooth in surfaces. This may be explained by faster solidification of the SS matrix than the SF matrix during the emulsification-diffusion process [15].

The internal morphology of the fractured microparticles was revealed from the SEM micrographs, as shown in Fig. 3. The SS, SF and blend microparticles showed a porous structure. This may be due to phase separation occurred within the emulsion droplets before particle solidification, as described in our previous work [15]. The porous structure could form when a non-solvent oil phase (ethyl acetate) diffused into each W/O emulsion droplet and reduced the solubility of the SS and SF, which then solidified and precipitated. However, the particle surfaces were continuous that completely covered the internal porous structures.

Table 1. Particle sizes of SS/SF blend microparticles

SS/SF blend ratio (w/w)	Average particle size (μm)[a]
100/0	52 ± 6
75/25	53 ± 4
50/50	46 ± 8
25/75	42 ± 5
0/100	37 ± 7

[a] *Determined from SEM images.*

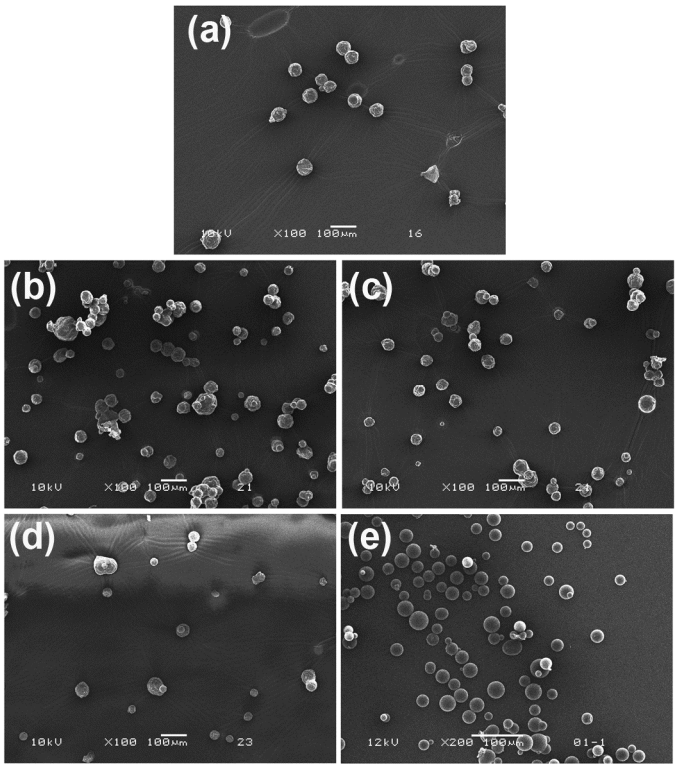

Fig. 1. SEM micrographs of blend microparticles prepared with SS/SF blend ratios of (a) 100/0, (b) 75/25, (c) 50/50, (d) 25/75 and (e) 0/100 (w/w). All bars = 100 μm

Average particle sizes of the microparticles were calculated from at least 100 diameters of the microparticles which obtained from several SEM micrographs. The results of particle sizes are reported in Table 1. The particle sizes of the SS and SF microparticles were 52 μm and 37 μm, respectively. The particle sizes of the blend microparticles slightly decreased as the SF blend ratio increased.

3.2. FTIR analysis of microparticles

FTIR spectroscopy was widely used to investigate conformation transition of both SS and SF matrices [5,12,15]. For this purpose, the amide I (C=O stretching) and II (C-N stretching) absorption bands are usually used to determine the secondary structure of the SS and SF. The FTIR spectra of the SS, SF and blend microparticles are shown in Fig. 4.

The amide I bands of SS and SF microparticles were 1650 and 1655 cm^{-1}, respectively. The amide II bands of SS and SF microparticles were 1646 and 1645 cm^{-1}, respectively. The FTIR analysis indicated that both SS and SF microparticles prepared from the W/O emulsification-diffusion method in this work consisted of dominate random coil form [5,12,15]. It can be seen that the wave number of amide I and II bands of the blend microparticles was lower than the SS and SF microparticles. This suggested the β-sheet content of the blend microparticles was higher than the SS and SF microparticles. In addition, these amide bands were shifted to lower wave numbers when the SF blend ratio was increased.

3.3. Dissolution of microparticles

Dissolution behavior of the microparticles was investigated in a phosphate buffer solution pH 7.4 at 37 °C for 24 h. The results of dissolution test are present in Fig. 5. It can be seen that the dissolution values of the plain SS and SF microparticles were 84% and 42%, respectively. This due to the SS matrix had higher hydrophilicity than that of the SF matrix. The dissolution values of blend microparticles steadily decreased as the SF blend ratio increased. The

results may be explained by the SS/SF blending induced conformational transition changed from a random coil (water soluble) to a β-sheet (water-insoluble) form, accorded to the previous FTIR results. Thus the dissolution of the blend microparticles could be tailored by adjusting the SS/SF blend ratio.

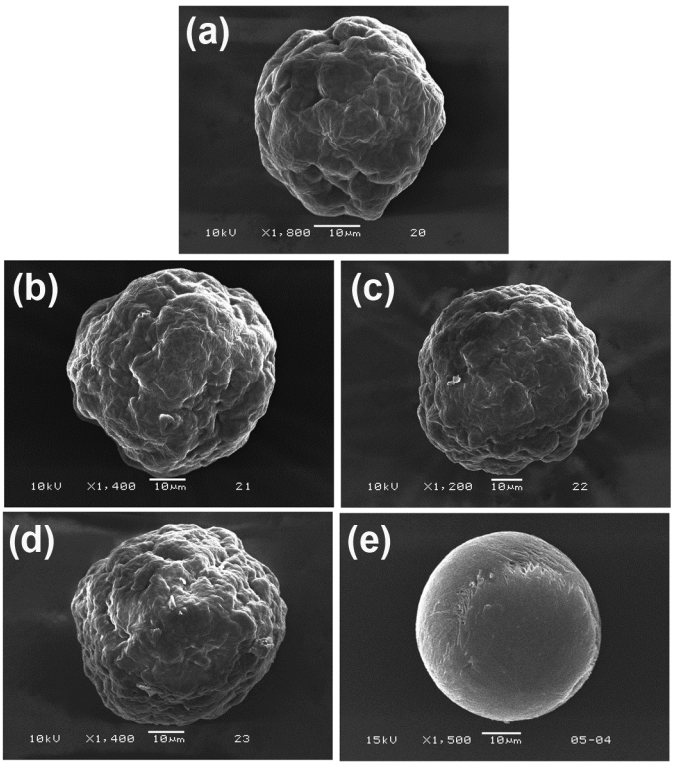

Fig. 2. Expanded SEM micrographs of blend microparticles prepared with SS/SF blend ratios of (a) 100/0, (b) 75/25, (c) 50/50, (d) 25/75 and (e) 0/100 (w/w). All bars = 10 μm

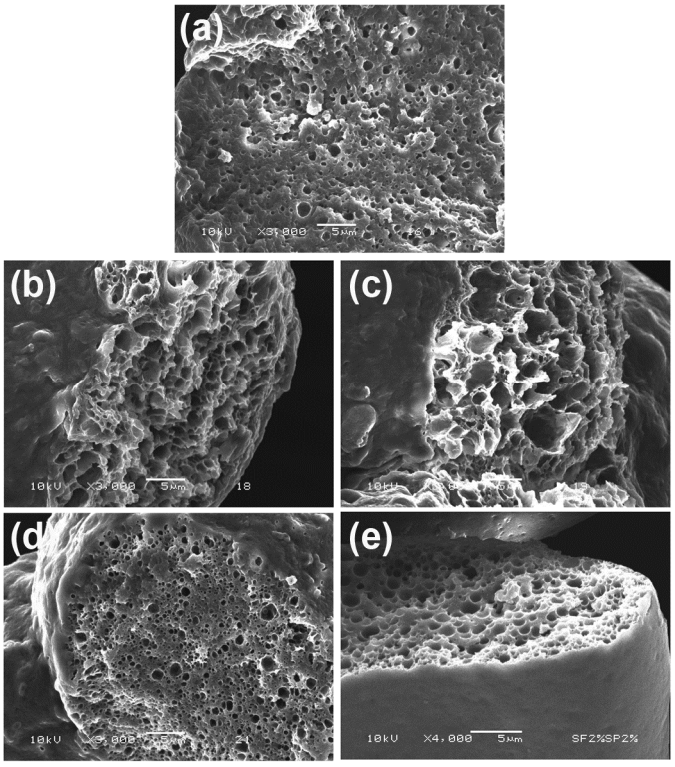

Fig. 3. SEM micrographs of fractured microparticles prepared with SS/SF blend ratios of (a) 100/0, (b) 75/25, (c) 50/50, (d) 25/75 and (e) 0/100 (w/w). All bars = 5 μm

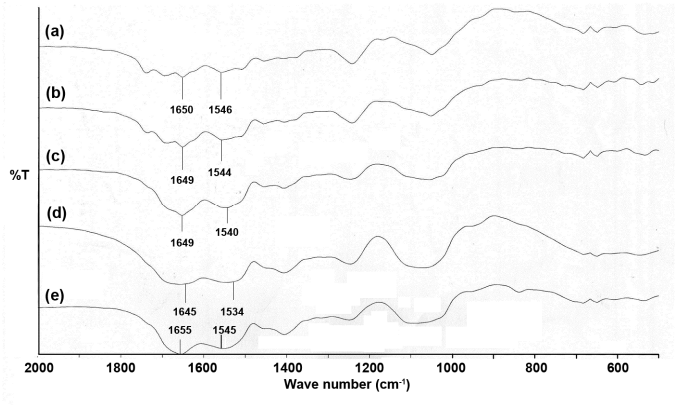

Fig. 4. FTIR spectra of blend microparticles prepared with SS/SF blend ratios of (a) 100/0, (b) 75/25, (c) 50/50, (d) 25/75 and (e) 0/100 (w/w)

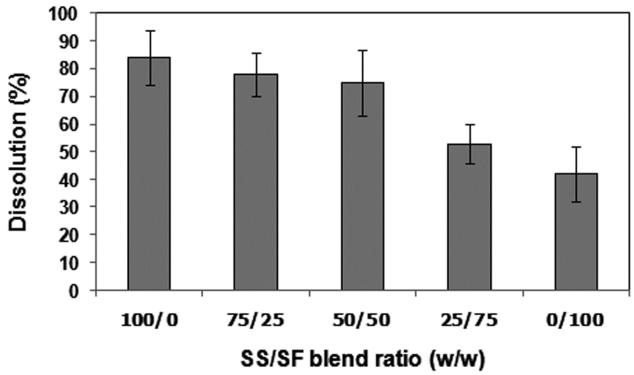

Fig. 5. Dissolution of blend microparticles prepared with different SS/SF blend ratios

CONCLUSION

The SS/SF blend microparticles with nearly-spherical shapes were successfully prepared by the W/O emulsification-diffusion method. The particle sizes of blend microparticles slightly decreased as the SF blend ratio increased. SEM of the microparticles showed that the surfaces of SS and SS/SF blend microparticles were rough, while the SF microparticles had smooth in surface. The SEM of fractured microparticles indicated the internal morphology of the all microparticles were porous structures. The FTIR analysis showed the conformational transition of the silk sericin changed from random coil to β-sheet form after blending with silk fibroin. The percentage of dissolution of the blend microparticles decreased with increasing SF blend ratios. These SS/SF blend microparticles have potential for use as controlled-release drug delivery systems for water-soluble drugs. The drug entrapment and drug release test of the SS/SF blend microparticles are under investigation.

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