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[Review]

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Biomedical Applications of Silk Protein

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Silk protein has been investigated by many researchers to apply to biomedical field. We reviewed biomedical applications of silk protein such as matrix of wound dressing and drug delivery system. Since silk fibroin/ poly (ethylene glycol) (PEG) semi-interpenetrating polymer networks showed good mechanical properties and wound healing phenomena, it can be used as wound dressing materials. Sericin nanoparticles prepared by conjugation with PEG and silk protein/ poloxamer mixture gel are expected to become a delivery as matrix for hydrophobic drug.

Key words : Silk protein, Wound dressing, Nanoparticles, Gel, Drug delivery system

Introduction

Silk protein produced by silkworm is composed of silk fibroin (SF) and silk sericin (SS). Over the centuries, SF has been used as highly valuable textile fibers due to its qualities of strength, elasticity, softness, absorbency, and affinity for dyes. Other applications of silk fiber include surgical suture due to its good physicochemical properties and relatively inert immune response for a long time. According to the Santin *et al.* (1999), SF is blood-compatible because fibroin bound lower levels of fibronectin than did polystyrene or poly (2-hydroxyethyl methacrylate), which has been used as biomedical materials. SF showed less inflammatory reaction than catgut used as bioabsorbable suture and poly (methyl methacrylate) used as ophthalmic biomaterials (Park, 1998). Silk protein has also been examined as a scaffold for cell culture (Minoura *et al.*, 1995; Tsubouchi, 1999).

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In this paper, we reviewed the biomedical applications of silk protein such as wound dressing and drug delivery system.

Wound dressing

SF has unique properties including good adherence with flexibility to wound bed, absorption of exudates, biocompatibility, biodegradability, and minimal inflammatory reaction. Moreover, SF has high water vapor and oxygen permeability and transparency in the wet state (Minoura *et al.*, 1990; Kweon *et al.*, 2001a). Therefore, SF can be used as wound dressing or ophthalmic materials. However, it has disadvantages like poor mechanical properties in sponge form. The mechanical properties of SF were enhanced by blending it with polysaccharide such as chitosan, cellulose or synthetic polymers such as poly (vinyl alcohol) (Freddi *et al.*, 1995; Chen *et al.*, 1997; Kweon *et al.*, 2001a).

SF/poly (ethylene glycol) (PEG) semi-interpenetrating polymer networks (SIPNs) was prepared to increase the mechanical properties of SF. PEG is one of the widely used and accepted biomaterial due to its water soluble, nontoxic, and non-immunogenic properties (Harris, 1992). PEG diacrylate used as a macromer was polymerized in the presence of SF to formulate SIPNs (Kweon, 2001b). Synthesis of SF/PEG SIPNs was confirmed using FT-IR spectra. The tensile strength and elongation at break of samples calculated from the load-elongation curves in the dry state are shown in Table 1. Both the tensile strength and elongation at the break of SIPNs were much higher than those of SF itself or SF/PEG blend. Also, those of SIPNs increased with an increase of PEG in the SIPNs due to the increase of crosslinking density of PEG network. In particular, the tensile strength of SIPNs (SF/PEG = 2/2 in weight) was 41 times higher than that of SF/PEG blend. The equilibrium water contents of SF and SF/PEG SIPNs against various ratios are shown in Fig. 1. The

Table 1. Tensile strength and elongation at break of SF, SF/PEG blend, and SF/PEG SIPNs (Kweon *et al.*, 2001b)

Sample (w/w)	Tensile Strength (N)	Elongation at Break (%)
SF	0.4	1.6
SF/PEG (2/2) blend	0.2	0.8
SF/PEG (2/2) SIPNs	8.2	6.0
SF/PEG (2/3) SIPNs	15	13
SF/PEG (2/3) SIPNs	26	48

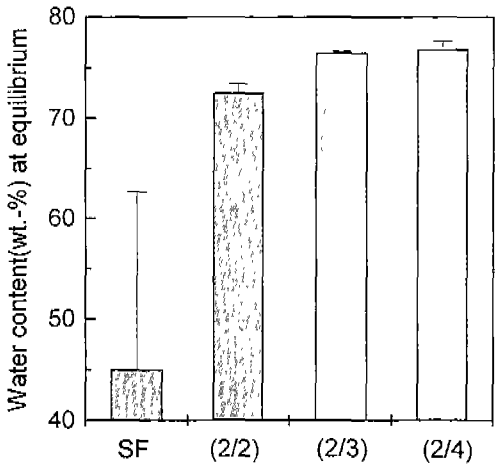
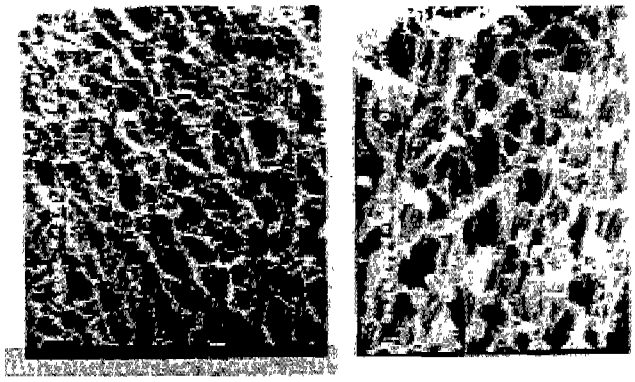
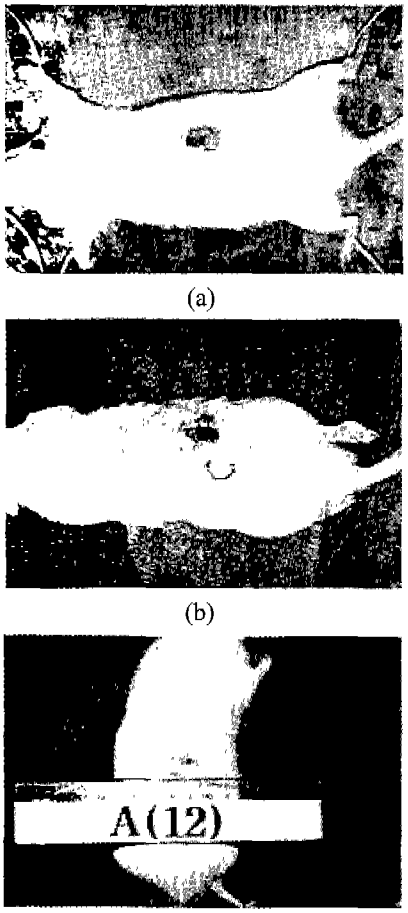


Fig. 1. Equilibrium water content of SF/PEG SIPNs against weight fraction of PEG.



(a) (b)

Fig. 2. SEM photographs of SF/PEG SIPNs: surface (a) and cross-section (b).



(a) (b) (c)

Fig. 3. Photographs of macroscopic appearances of wound contraction covered with wound dressing at 0 (a), vaseline gauge after 12 days (b), and SF/PEG SIPNs after 12 days (c).

Table 2. Contents of collagen produced in wound area at 12 days after dermal graft by using image analysis (Yeo *et al.*, 2000)

Dermal Graft Composition	Collagen Content (%)
Control (non-treated)	2.42 ± 0.32
Chitosan	23.87 ± 7.04
Fibroin	46.05 ± 4.53
PVA + Chitosan	30.54 ± 3.25

Values are the mean ± SD (n=5).

water contents of SF/SIPNs were higher than SF itself due to the hydrophilic properties of PEG. But the effect of PEG content on the water content of the SIPNs was not much different due to the increased crosslinking density of PEG network with an increase of PEG in the SIPNs. The morphology of SF/PEG SIPNs (Fig. 2) shows interconnected pores. To observe the wound healing phenomena of SF/PEG SIPNs, photographs of macroscopic appearance of wound contraction are shown in Fig. 3. The wound covered with SF/PEG SIPNs was almost healed after 12 days. However, vaseline gauge as the control is not very well recovered after 12 days. From this result, SF/PEG SIPNs have a facilitating effect of wound. According to the degree of collagen generation depending on SF as wound dressing (Sugihara *et al.*, 2000; Yeo *et al.*, 2000), SF has been turned out to facilitate the secretion of

collagen. Table 2 is the collagen content covered with wound dressings after 12 days. Collagen content covered with SF shows the highest value (46.05%) among them. From this result, the wound healing effect of SF/PEG SIPNs was attributed to collagen secretion of SF in the SIPNs.

Cytotoxicity of SF/PEG SIPNs was estimated by the cell proliferation using pKH26 membrane dye and cell cycle software. Proliferation of L929 cells between medium as control and SF/PEG SIPNs was not much different according to the cell cycle, indication of non-cytotoxicity of SF/PEG SIPNs.

Conclusively, mechanical properties of the SF were enhanced by formation of SIPNs with PEG and SF/PEG SIPNs showed faster wound contraction than vaseline gauge as a control. Also, cytotoxicity was not found in the SF/PEG SIPNs. Therefore, SF/PEG SIPNs can be used as wound dressing materials.

Drug Delivery System (DDS)

Nanoparticles and hydrogel have been investigated as matrix for DDS by many researchers (Hanawa *et al.*, 1995; Peppas *et al.*, 2000). Nanoparticles are colloidal particles of a size below 1 um and have been studied in various fields of life sciences (e.g., separation techniques, DDS, and cosmetics). The nanoparticles based on polymeric micelles are stable in aqueous medium and can dissolve hydrophobic drugs in their inner core (Kataoka *et al.*, 1993). Advantages of nanoparticles for applications in pharmaceutical technology are easy purification, sterilization, drug targeting possibility, and sustained release action (Allemann *et al.*, 1993; Cho *et al.*, 1997).

Gels, especially hydrogels, are water-swollen polymers having a water content ranging from 30 to 90 wt.%. Because of their highly swollen nature, hydrogels offer a possibly effective and convenient delivery vehicles to administer large molecular weight protein- and peptide-based drugs (Ranade and Holliger, 1995).

Sericin nanoparticles

In general, silk sericin considered as a waste in textile industry was removed through a degumming process. However, sericin has cosmetic activity (Voegeli *et al.* 1993; Engel *et al.*, 1987; Kato *et al.*, 1998) including a good retention ability of water on the cutaneous surface due to the presence of several hydroxyl groups. But it is difficult to prepare sericin nanoparticles due to unstability in water and insolubility in organic solvents. To overcome the above problem, self-assembled sericin nanoparticles were prepared through PEGylation (Cho *et al.*, 2001).

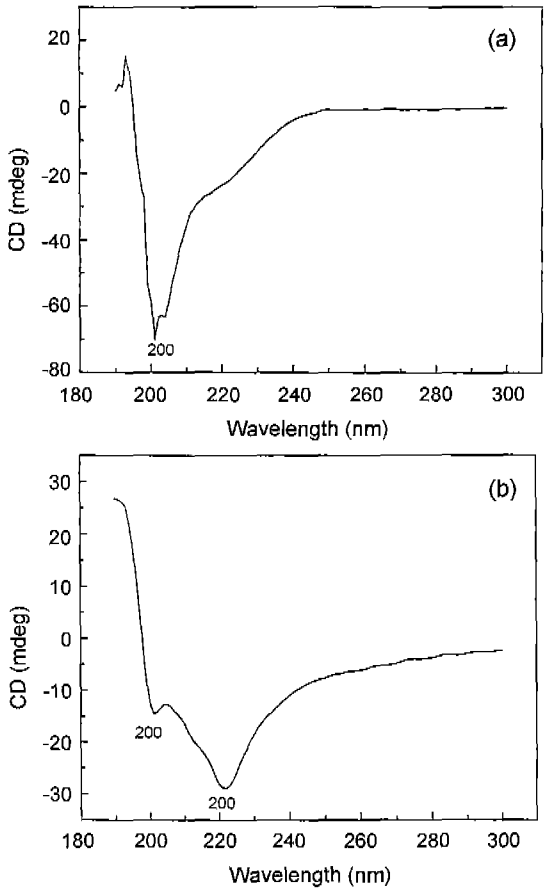


Fig. 4. CD spectra of sericin (a) and sericin-PEG conjugate (b).

The synthesis of sericin/PEG conjugate was confirmed using ^{1}H-NMR and FT-IR spectrometer. The conformation of sericin in the sericin/PEG conjugate was examined through CD spectrometer and the results are shown in Fig. 4. The CD spectrum of sericin itself shows a peak at 200 nm trough, which is an indication of the random coil conformation. On the contrary, that of sericin/PEG conjugate exhibits negative peaks at 200 and 220 nm, indicating that the secondary structure of sericin was changed from random coil to a-helix after the conjugation of sericin with PEG. The morphology of sericin nanoparitcles (Fig. 5) is a spherical type, and average particle sizes measured using particle size analyzer are ca. 150 nm (Table 3).

From the above results, sericin nanoparticles was prepared by conjugation with PEG and will be expected to be delivery matrix for hydrophobic drug.

Fibroin gel

Gelation behavior of SF was dependent on the concentration of SF, temperature, and pH (Min *et al.*, 1998, Lee *et al.*, 1999). The sol-gel transition of SF is irreversible because the hydrogen bonds between fibroin molecules are too strong to be broken at the mild conditions.

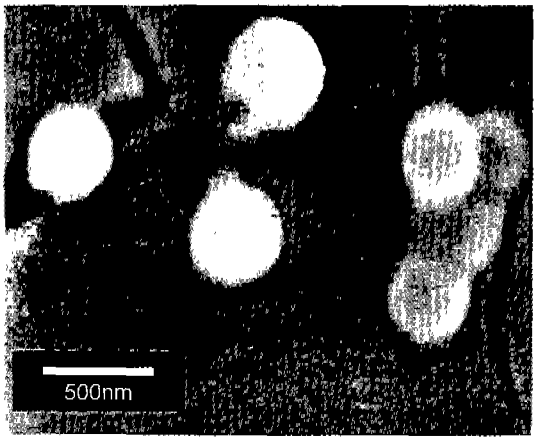


Fig. 5. SEM photographs of the sericin-PEG conjugate.

Table 3. Particle size distribution of sericin nanoparticles (Cho *et al.*, 2001)

Sample	Particle Size (nm)		
	Intensive Avg.	Number Avg.	Weight Avg.
SS/PEG conjugate	197.3 ± 46	149.0 ± 28.8	168.8 ± 38.2

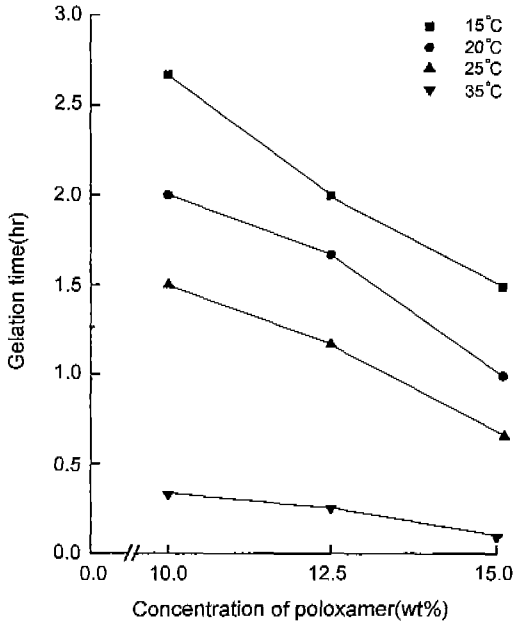


Fig. 6. Gelation time of aqueous SF solution against concentration of poloxamer according to the various temperature (conc. of SF: 2.98 wt.%).

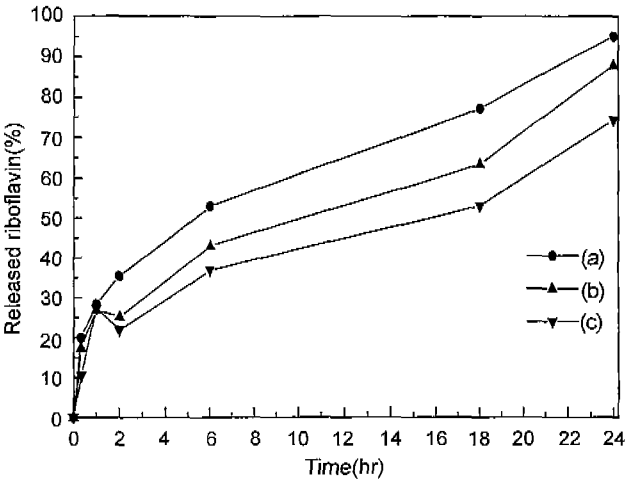


Fig. 7. Riboflavine delivery profiles from SF/poloxamer gel: (a) 3.0/10 (w/w), (b) 3.0/12.5, and (c) 3.0/15.

Therefore, the fibroin hydrogels have a limitation for the application of DDS. Poloxamer is widely used for DDS due to its good biocompatibility, little toxicity, rapid reversible sol-gel transition behavior according to the temperature and concentration (Redhead *et al.*, 2001; Suha and Jun, 1996). The gelation time of SF in the presence of poloxamer was significantly decreased with an increase of temperature, poloxamer, and concentration of SF (Fig. 6). In particular, the gelation occurred within 5 min at pH 7, 15 wt% of poloxamer, and 2.98 wt% of fibroin. It might be thought that gelation was accelerated through dehydration of water around fibroin molecules by hydrophilic poloxamer, resulting in the conformational change of SF from random coil to b-structure. Interestingly, the gelation of SF at pH value of 3.8 (isoelectric point) did not take place in the presence of poloxamer, whereas the gelation of SF itself occurred. It might be supposed that the gelation of poloxamer was affected by the citric acid used to adjust pH value of 3.8 as the similar behavior of sodium dodecyl sulfate and the interaction between SF and poloxamer was weakened by the citric acid. It is reported that the gelation of poloxamer is reversible with temperature change through the hydrophobic interaction of poly (propylene oxide) in the copolymer, whereas the gelation of SF is irreversible with temperature change through the intermolecular hydrogen bonds. The gelation of the SF/poloxamer mixture system became reversible by the change of hydrophobic interaction between poloxamer and SF, and hydrogen bonds among SF molecules. But it may be regarded that the presence of poloxamer 407 micelles (CMC 0.08 wt% at 30°C) does not interfere gelation process of the SF but enhances the gelation of it. Release profiles of riboflavin as a model drug from SF/poloxamer hydrogel are shown in Fig. 7. As expected, release rate of riboflavin was decreased with an increase of poloxamer.

In conclusion, the gelation of SF was accelerated with an increase of poloxamer concentration and temperature (Kang *et al.*, 2000). Release of drug can be controlled by

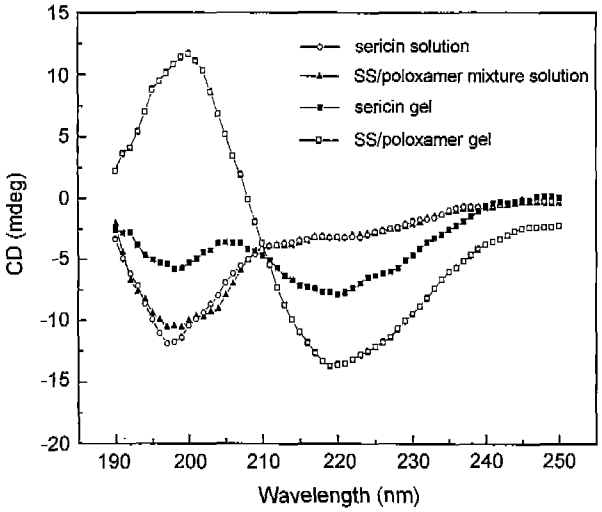


Fig. 8. CD spectra of silk sericin solution and gel mixed with poloxamer.

SF/poloxamer composition. Therefore, this system may provide drug delivery vehicle.

Sericin gel

Sericin is a molecule with lower weight and lower crystallinity, and more hydrophilic than those of SF. Sericin solution is slowly transformed into gel with time. Sol-gel transition of sericin is reversible, whereas it is different in SF (Hirabayashi *et al.*, 1990). The gelation behavior of sericin in the presence of poloxamer showed similar tendency of SF. However, the sol-gel transition of sericin in the presence of poloxamer became irreversible. Considering these results, sol-gel transitions of silk proteins, fibroin and sericin, in the presence of poloxamer is different. When poloxamer was added into the sericin solution, sericin molecules were closed to each other and packed. Since sericin is more hydrophilic than SF, inter/intra-molecular interactions between sericin molecules affected more severely by hydrogen bonding than hydrophobic interaction. Therefore, sericin molecules would form β-structure in the presence of poloxamer. CD spectra of sericin solution and gel mixed with poloxamer are shown in Fig. 8. Sericin solution showed a negative peak at 198 nm attributed to random coil, while sericin mixed with poloxamer solution showed another shoulder peak at about 205 nm. These results indicate that the conformation of sericin was affected by the presence of poloxamer. Sericin gel showed two negative peaks at 198 and 218 nm and sericin/poloxamer mixture gel showed a negative peak at 218 nm, attributed to β-structure.

In conclusion, the gelation of sericin was affected by the poloxamer. In particular, the sol-gel transition of sericin/poloxamer mixture solution became irreversible, whereas the gelation of sericin itself was reversible (Kweon *et al.*, 2000).

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