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Effect of silk fibroin molecular weight on physical property of silk hydrogel

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ABSTRACT

Silk hydrogel has recently received great attention for its excellent biocompatibility. The property of silk hydrogel is, however, not only hardly controlled but very limited due to non-variability of silk fibroin (SF) molecule. In this study, alkaline hydrolysis was utilized to manipulate the silk hydrogel properties. By regulating the hydrolysis time (10–180 min), a broad molecular weight range of SF was obtained (263.1–82.7 kDa) Gel point increased with a decrease of SF molecular weight. The change of molecular weight of SF also greatly affected the physical properties (i.e., swelling ratio, shear modulus, transparency) as well as cell adhesion of SF hydrogels. As a result of structural analysis, the molecular weight of SF played a crucial role in the construction of microscopic structure of SF hydrogel. These findings indicate that SF hydrogels of variable physical properties can be fabricated based on molecular weight control for diverse purposes in biomedical engineering.

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1. Introduction

Silk fibroin (SF) is a major protein, which plays a critical role in both structural feature and mechanical property of silk cocoons. In raw silk fibers, two SF strands are covered with a gum-like protein, silk sericin (SS), which is easily removed by weak alkali hot water [1]. To date, it has been widely reported that SF shows excellent biocompatibility as well as superior mechanical property in biomedical applications [2–7]. Besides, the facile recrystallization by alcohol treatment inducing the physical cross-linking via intra- and inter-molecular hydrogen bonding of hydrophobic segments of SF is generally exploited in the fabrication process [8,9]. Such a process is very useful in biomedical material fabrications (e.g., tissue engineering scaffold, drug carrier) since this process does not cause any harmful chemical species damaging living organisms.

SF can be fabricated into various forms (e.g., film, nanofiber, sponge, hydrogel) [10]. Especially, SF hydrogel has been recently received great attention in tissue engineering field. The network structure of SF hydrogel retains the large amount of water with inter-connected porous structure. This structure provides the physiologically stable condition for cell survival [11,12]. Also, porous three-dimensional (3D) structure of SF hydrogel is an appropriate microenvironment in which cells reside allowing exchanging nutrients and wastes. Therefore, SF hydrogel shows very excellent biological properties compared to synthetic polymer-based hydrogel (e.g., poly(ethylene glycol) [13], poly(vinyl alcohol) [14]) as a 3D cell niche. The properties of SF hydrogel are affected by a wide variety of processing parameters, such as concentration of SF solution [15,16], incubation temperature [15], vortexing time [7], and ultra-sonic power [12], The concentration is a major factor to determine physical properties of SF hydrogel [15,16]. Generally, the higher concentration results in the stiffer hydrogels with the shorter gelation time. The gelation behavior is also largely dependent on the incubation temperature [15]. For example, the SF hydrogel formed at a higher temperature (~50 °C) mostly has a higher Young's modulus than that formed at a lower temperature (~4 °C). The effect of amplitude of shear force was also investigated. Wang et al. has shown that the amplitude of ultra-sonication affects the mechanical properties of SF hydrogel [12]. Yucel et al. reported the vortexing time effect on the shear modulus of SF hydrogel [7]. Although the significant few studies to manipulate SF hydrogel properties were reported, none of processing variables could elicit the change of a wide range in mechanical as well as physical properties except the concentration of the precursor SF solution.

Generally, the molecular weight of polymer composing the

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network structure of hydrogel is a crucial factor that determines the gel properties (i.e., modulus, swelling ratio, permeability) [17–19]. Both cross-linking density and mesh size of hydrogel are largely dependent on the polymer chain length regardless of cross-linking methods (i.e., chemical and physical cross-linking) [20,21]. Nevertheless, the effect of molecular weight of SF on hydrogel fabrication has not been explored. Hence, in this study, we conducted the heat-alkaline treatment (HAT) during the SF dissolution step to control the molecular weight of SF as a one-pot process. This method is not only simple but also suitable to obtain a high yield of hydrolyzed SF. In contrast, proteinase treatment needs relatively delicate process including enzyme removing process and causes sever mass loss of product in spite of high efficiency [22]. Then, we formed SF hydrogel by ultra-sonication, followed by physical and mechanical property analyses to investigate the effect of molecular weight of SF on the hydrogel formation. Especially, we tried to focus on the change of the microscopic structure of SF hydrogel according to molecular weight variation, which allows the wide range property control of the hydrogel. Finally, it was demonstrated that such a property manipulation of SF hydrogel can be successfully utilized in tissue engineering field by human mesenchymal stem cell (hMSC) culture on SF hydrogels.

2. Experimental

2.1. Materials

To remove silk sericin, *Bombyx mori* cocoons were boiled in 0.3% (w/v) sodium oleate and 0.2% (w/v) sodium carbonate cocktail solution at 100 °C for 1 h. The SF aqueous solutions were obtained by using two different dissolving methods. For LiBr-dissolution method, the degummed cocoons were dissolved in 9.3 M LiBr solution at 80 °C for 30 min. To hydrolyze SF, 0.6 M sodium hydroxide aqueous solution was directly added to the SF solution at a volume ratio of 1-to-5. Then, the final concentration of sodium hydroxide became 0.1 M in the SF solution. To control SF molecular weight, the hydrolysis time was varied from 10 to 180 min, followed by subsequent dialysis against de-ionized water using cellulose acetate dialysis tube (MWCO: 12,000–14,000 Da) for 3 days.

For $CaCl_2$-dissolution method, the degummed cocoons were dissolved in a ternary solvent of a $CaCl_2/H_2O$/EtOH (molar ratio 1/8/2) at 80 °C for 5 min instead of LiBr solution. The hydrolysis was directly performed at 80 °C for 10–120 min. The detail preparation conditions and sample ID were listed in Table 1 and Table S1. After dialysis, SF solutions were centrifuged at 3000 g for 10 min to remove insoluble aggregates. The final concentrations of SF solutions were in the range of 3.5–4% (w/v) and each solution was diluted to 3% (w/v) concentration. The prepared SF solutions were stored at 4 °C until gel fabrication.

Sodium oleate, lithium bromide 1-hydrate, and calcium chloride were purchased from Tokyo Chemical Industry, Kanto Chemical, and Yakuri, respectively. The other chemicals were purchased from Sigma–Aldrich without further purification.

Table 1
Sample ID and preparation conditions of alkali hydrolyzed SF solutions using LiBr-HAT method.

Sample ID	Dissolution condition	Dissolution time (min)	Hydrolysis condition	Hydrolysis time (min)
L0	Solvent: 9.3 M	30	Solvent: 0.1 M	0
L10	LiBrTemperature:		NaOH	10
L30	80°CLiquor ratio: 1 g/5 mL		Temperature:	30
L90			80 °C	90
L180				180

2.2. SF hydrogel fabrication

To initiate the gelation of SF solution, ultra-sonication was performed on 3% (w/v) SF aqueous solution of different molecular weights using an ultra-sonic processor (VCX-130, SONICS, USA) at 25% amplitude for 3 min. The treatment was conducted in an ice chamber to prevent the heat elevation during ultra-sonication. Then, ultra-sonicated SF solution was filtered and incubated at 60 °C for 2 days.

2.3. Gel filtration chromatography

The molecular weight of SF was measured by gel filtration chromatography (GFC) (AKTA Purifier, GE Healthcare, USA) with Superdex 200 10/300 GL column (GE Healthcare, USA). 0.5 mL of 3% (w/v) SF solution was added to 4 mL of 6 M urea aqueous solution, followed by filtration with 0.2 μm membrane. For the measurement, 300 μL of sample solution was injected and 1.5 column volume of 4 M urea was eluted at a constant flow rate of 0.5 mL/min. The elution of SF was detected at 280 nm. The molecular weight of SF was determined by a calibration curve, which was obtained by a standard globular protein kit (Gel Filtration Cal Kit High Molecular Weight, GE Healthcare, USA).

2.4. Visible light transmittance measurement

3% (w/v) SF solution was transferred into polystyrene UV/Vis spectrometry cuvette and each cuvette was completely sealed. The absorbance was measured in the range between 400 and 700 nm by using a UV/Vis spectrometer (OPTIZEN POP, Mecasys, Korea). The path length was fixed at 10 mm. To measure the turbidity, SF hydrogels were formed in the same cuvette by ultra-sonication and subsequent incubation at 60 °C for 2 days.

2.5. Rheometry

To determine the gelation time (gel point), shear elastic and loss moduli (G′ and G″) were measured by a rheometer (HAAKE MARS III, Thermo Fisher Scientific, Germany) over the SF solution incubation time after ultra-sonication. The measurement was performed using a time-sweep oscillatory mode (strain: 5%, frequency: 1 Hz, gap size: 0.1 mm) with a parallel plate geometry (Dia.: 35 mm). All the solutions were stored at 60 °C before measurement. Gel point was determined when G′ surpassed G″. To measure the equilibrium shear elastic modulus, SF hydrogel slabs were formed, followed by subsequent swelling in pH 7.4 PB S at 37 °C for 24 h. The swollen SF hydrogel was punched out using a biopsy punch (8 mm). Then, shear elastic modulus (G′) was measured by the rheometer using a strain-sweep oscillatory mode (strain: 0.1–10%, frequency: 1 Hz, gap size: 2.5 mm) with a parallel plate geometry (8 mm). After the measurement, G′ was determined from the linear viscoelastic region.

2.6. Swelling behavior

To determine gel fraction of SF hydrogel, each gel was cut into a square slab (5 mm × 5 mm × 0.1 mm) and dried at 60 °C for 24 h in vacuum right after the fabrication, followed by original dry-weight (W_{d0}) measurement. The dried gels were incubated in de-ionized water at 37 °C for 24 h and washed several times. The swollen gels were then re-dried in vacuum for 24 h and its dry-weight (W_{d1}) was measured. The gel fraction was obtained by following Eq. (1).

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