Research Article

Comparison of Gelation Time and Polyalcohol Effect on Hydrogels from Domestic and Wild Silk Fibroins

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Silk fibroin (SF) hydrogels were obtained from both domestic (*Bombyx mori*) and wild (*Antheraea pernyi*) silkworms from aqueous silk fibroin solutions at room temperature. The gelation time of the *Antheraea pernyi* (*A. pernyi*) SF solution was significantly shorter than that of the *Bombyx mori* (*B. mori*) SF solution. The secondary structures of the two kinds of hydrogels were also compared. In order to further reduce the gelation time, various amounts of polyethylene glycol (PEG) were blended with the silk fibroins of *A. pernyi* and *B. mori*. The gelation time of both *A. pernyi* SF and *B. mori* SF decreased with the increased amount of PEG. After freeze-drying, the hydrogels were characterized through X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and Raman spectroscopy. Results showed that the addition of polyalcohol did not change the main secondary structure of the hydrogels. However, the addition of polyalcohol did reduce the gelation time and triggered additional formation of β -sheets.

1. Introduction

Hydrogels can maintain a distinct three-dimensional porous structure with mechanical and structural properties similar to that of many natural tissues and extracellular matrices (ECMs) and can be delivered in a minimally invasive manner [1]. Therefore, hydrogels are of interest for many biomedical applications such as tissue engineering scaffolds, controlled drug release devices, and biosensors [2]. Hydrogels can be made of either synthetic materials or naturally derived materials [3]. Among naturally derived materials, it is agreed that silkworm silk fibroin (SF) is one of the most promising biomaterials because of its excellent biocompatibility, biosafety, controllable biodegradation rates, processability, and mechanical properties [4]. Silkworm silk fibroin is excreted by domestic (*B. mori*) and wild (*A. pernyi*) silkworms. Domestic SF hydrogels are of interest for use as bone-filling biomaterials, wound dressing, and so on and have proved to be promising for healing efficacy [5–10]. Compared with *B. mori* SF, *A. pernyi* SF, the most familiar wild SF,

has more advantages in terms of chemical activity and high-affinity interactions with mammalian cells [11]. *A. pernyi* SF is rich in amino acids having polarity and ionogenic pendant groups, which are the potential reactive sites of chemical reactions. In terms of amino acids, *A. pernyi* SF has more Ala, Asp, and Arg and less Gly than does *B. mori*. Further, *A. pernyi* SF also has abundant alkaline amino acids (Arg and His) and the tripeptide sequence Arg-Gly-Asp (RGD), which is known to be a cell integrin receptor and to mediate special interactions between mammalian cells. It has been demonstrated in some research that *A. pernyi* SF performs better in terms of cell adhesion, growth, and phenotypic maintenance than does *B. mori* SF [12]. Also, *A. pernyi* has bioproperties similar to collagen, such as cell adhesion, growth, and proliferation. Therefore, it is supposed that biomedical materials made of *A. pernyi* SF have better bioproperties than do those made of *B. mori* SF. However, biomaterials made of *A. pernyi* SF have not been studied as widely as those made of *B. mori* SF, especially in hydrogels. Because of their improved properties and better performance, introducing wild SF

hydrogels as a potential biomaterial in tissue engineering is very necessary. Therefore, hydrogels made of *A. pernyi* SF were prepared and studied in the present study.

Through reviewing research articles about *B. mori* SF hydrogels, we found that the gelation time of SF hydrogels is excessively long unless nonphysiological treatments (such as low pH, high temperature, or additives) are considered [6, 13–17]. The question we want to answer is do the *A. pernyi* SF hydrogels have a similar problem? This question prompted us to search for effective ways to reduce the gelation time of *A. pernyi* SF solutions. In addition to the methods of γ -ray irradiation, ultrasonication, and shearing, blending auxiliary materials like polyalcohol with SF has been considered an effective method to reduce gelation time because it induces the transition of SF from random coil and α -helix structures to β -sheet structures [18]. Among polyalcohols, polyethylene glycol (PEG) is a linear polymeric material that is polymerized by glycol monomers. The presence of abundant oxethyl groups in the PEG molecule that are capable of forming hydrogen bonds with water makes PEG a highly water-soluble material. PEG is also a nontoxic, nonimmunogenic, and amphipathic polymer, and it possesses a variety of properties pertinent to biomedical and biotechnical applications [18–20]. Thus, adding PEG to *A. pernyi* SF solutions was selected as a method for reducing gelation time in this study. *B. mori* SF hydrogels with and without PEG were also prepared as control samples. *A. pernyi* and *B. mori* SF hydrogels were characterized after freeze-drying through X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and Raman spectroscopy. The effect of PEG on gelation time and the final secondary structure of the hydrogels were also studied.

2. Materials and Methods

2.1. Preparation of SF Aqueous Solutions. The process of preparing SF aqueous solution is described elsewhere [11, 21]. To be specific, cocoons of *A. pernyi* were boiled for 30 min in a 0.25 wt% Na_2CO_3 aqueous solution to remove sericin. Degummed *A. pernyi* SF fibers were rinsed thoroughly with pure water. After oven drying (60°C), they were dissolved in $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (the liquor ratio was 1:10) at 105°C for 5 h. Then, the mixed solution was dialyzed against flowing distilled water for 96 h to remove salt. The concentration of *A. pernyi* SF aqueous solution was about 1.78 wt%, and it was then concentrated to 3.0 wt% through air drying.

B. mori silks were degummed by being boiled for 30 min in a 0.06 wt% Na_2CO_3 aqueous solution. After being rinsed and oven dried (60°C), the degummed *B. mori* SF fibers were dissolved in the triadic solvent $\text{CaCl}_2 \cdot \text{CH}_3\text{CH}_2\text{OH} \cdot \text{H}_2\text{O}$ (mole ratio = 1:2:8) at 72°C for 1 h with stirring; the liquor ratio of the triadic solvent was 1:10. This solution was dialyzed against flowing distilled water for 96 h. The final concentration of *B. mori* SF solution was also about 3.0 wt%.

2.2. Preparation of SF Hydrogels. PEG 600 was added to the two 3.0 wt% SF solutions in ratios of 0, 50, 100, 150, 300, 500, 700, and 900 wt% (PEG/SF) and gently stirred at

room temperature to disperse the PEG homogeneously. The mixed solutions were then left to stand at room temperature. The formation of gels was determined by inserting a glass capillary into the mixed solutions; when the liquid level in the glass capillary stopped rising, the gelation time of each sample was recorded. In order to measure their structure, the hydrogels were then frozen at -40°C and lyophilized.

2.3. X-Ray Diffraction (XRD). The hydrogel samples were made into powders and measured by X-ray diffractometer (X'PERT PRO MPD) with $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$). The scanning speed was $2^\circ/\text{mm}$. The diffraction intensity curves with 2θ from 5 to 45° were obtained.

2.4. Fourier Transform-Infrared (FT-IR) Spectroscopy. The hydrogel samples were made into powders, using KBr tabletting to prepare specimens. An FT-IR spectrophotometer (Nicolet Avatar-IR 360) was used to analyze the samples. All spectra were recorded by absorption mode at 2 cm^{-1} interval and in the wavelength range of $1800 \sim 400 \text{ cm}^{-1}$.

2.5. Raman Spectroscopy. Raman spectra were obtained by Raman spectrometer (LabRam-1B) at a wavelength of 632.8 nm , the resolution of which was 2 cm^{-1} . The scanning time for solid specimens was 200 s.

3. Results and Discussion

3.1. Gelation Time. It can be seen in Figures 1 and 2 that the original gelation time of *A. pernyi* SF was 69.3 hours, which is much shorter than that of *B. mori* SF (gelation time was 288.6 h). Pure *A. pernyi* SF can also gelate quicker than that of *B. mori* SF with 500 wt% PEG (gelation time is 103.1 h). The structure of the SF hydrogels was stabilized due to the formation of β -sheets, which served as physical cross-links [22, 23]. *A. pernyi* SF is rich in amino acids having polarity and ionogenic pendant groups, which more easily forms reactions with other polar molecules and functional groups. The movements and interactions between the SF molecules themselves and the PEG in the *A. pernyi* SF aqueous solution would be more violent than those in the *B. mori* SF aqueous solution. The transition of β -sheets would be therefore more rapid and much easier.

The gelation time of *A. pernyi* SF decreased with the addition of PEG, and it decreased to 38.0 h when the amount of PEG was 500 wt%. A small amount of PEG (only 50%) can reduce the gelation time of *A. pernyi* SF, whereas the gelation time of *B. mori* SF required the addition of as much as 300 wt% PEG to significantly reduce the gelation time. PEG was highly water-soluble once it was blended with the SF aqueous solution, forming abundant hydrogen bonds and breaking the hydration layer around the SF molecules. The molecules of SF and PEG started to move and collide with each other, which accelerated the aggregation of SF molecules and triggered the breaking and rebuilding of hydrogen bonds. Through this process, random coil and α -helix structure were induced to transform into β -sheets.

FIGURE 1: The relationship between gelation time of *A. pernyi* silk fibroin and PEG amount.

FIGURE 2: The relationship between gelation time of *B. mori* silk fibroin and PEG amount.

Figure 2 shows that preparing *B. mori* SF is time-consuming, which is a drawback for the fabrication of SF hydrogels. However, in this study, it was found that *A. pernyi* SF can gelate much quicker than *B. mori* SF. Besides, adding a small amount of PEG (only 50%) can further reduce the gelation time of *A. pernyi* SF. Hydrogels from *A. pernyi* SF also have better bioproperties and are more advantageous in terms of high-affinity interactions with cells; the shorter preparation time is another advantage for the use of these hydrogels in potential biomedical applications.

3.2. X-Ray Diffraction. The formation of β -sheets is an important issue in stabilizing the structure of SF hydrogels. Secondary conformation of hydrogels from pure *A. pernyi* SF, pure *B. mori* SF, and SFs blended with PEG are shown in the XRD curves in Figure 3. For the diffraction peaks of silk fibroin, please see [21, 24, 25]. In Figure 3(a), the diffraction peaks of pure *A. pernyi* SF hydrogels, shown in curve (A), appear around 16.5° , 20.2° , 24.9° , and 30.9° and are attributed to β -sheet structure. The diffraction peaks at 16.5° , 24.9° , and 30.9° become wider (curve (B)) and then disappear (curve (C)), while the main diffraction peaks at 20.2° remain. These results indicate that the secondary structure of *A. pernyi* SF hydrogels contains mainly β -sheets; however, with the addition of PEG (100 wt% and 300 wt%), the diffraction peaks of the hydrogels are covered up by those of PEG. A similar phenomenon is observed in Figure 3(b), in which the secondary structure of pure *B. mori* SF (curve (A), with diffraction peaks at 9.1° , 20.7° , and 24.7°) consists

mainly of β -sheets. With the addition of PEG, diffraction peaks at 9.1° and 24.7° are covered up, while peaks at 20.7° remain (curves (B) and (C)). The diffraction curves of the hydrogels with other blending ratios of SF and PEG were the same, so the data for these samples are not shown; only representative curves were selected for analysis. Figure 3 indicates that the secondary structure of both *A. pernyi* SF and *B. mori* SF are mainly β -sheets, and the addition of PEG did not influence the crystalline structure of the SF hydrogels.

3.3. FT-IR Spectroscopy. In order to prove the results of X-ray diffraction, FT-IR spectra were also obtained in this study, the results of which are shown in Figure 4. For the characteristic peaks of silk fibroin in FT-IR spectra, see [25–28]. In Figure 4(a), relatively strong characteristic peaks appear in curve (A) at 1631 cm^{-1} (amide I), 1521 cm^{-1} (amide II), 1240 cm^{-1} (amide III), 965 cm^{-1} (amide IV), and 700 cm^{-1} (amide V), which means that pure *A. pernyi* SF hydrogels contain large amounts of β -sheets. Meanwhile, weak characteristic peaks appear in curve (A) at 881 cm^{-1} (amide IV) and 620 cm^{-1} (amide V), which indicates that a small amount of α -helix structures coexist with the β -sheets. With the addition of PEG, the characteristic peaks attributed to β -sheets become stronger (curves (B) and (C)), while those attributed to α -helix structures become smoother (curves (B) and (C)). A similar phenomenon is observed in Figure 4(b), in which the secondary structure of pure *B. mori* SF (curve (A)) consists of a large amount of β -sheets (1635 cm^{-1} , 1620 cm^{-1} , 1234 cm^{-1} , and 700 cm^{-1}) and a small amount of α -helix structures (610 cm^{-1}). With the addition of PEG, the characteristic peaks attributed to β -sheets become stronger (curves (B) and (C)), while those of the α -helix (610 cm^{-1}) disappear. The above phenomena indicate that the addition of polyalcohol triggered the transition of more β -sheets and that the main secondary structure in *A. pernyi* and *B. mori* SF hydrogels remains β -sheets.

3.4. Raman Spectroscopy. In order to confirm the results of X-ray diffraction and FT-IR, laser Raman spectroscopy of *A. pernyi* and *B. mori* SF hydrogels with added PEG at ratios of 0 and 300 wt% were detected, as shown in Figure 5. For the characteristic peaks of silk fibroin in Raman spectra, see [29]. In Figure 5(a), strong characteristic peaks in the Raman spectra of pure *A. pernyi* SF hydrogels (curve (A)) show around 1669.07 cm^{-1} , 1223.62 cm^{-1} , and 1093.78 cm^{-1} , which are attributed to β -sheets, while relatively weak characteristic peaks appear around 1657.88 cm^{-1} , 1646.67 cm^{-1} , 1269.36 cm^{-1} , and 907.29 cm^{-1} , which are attributed to the α -helix. Similarly, the secondary structure of pure *B. mori* SF hydrogels contains large amounts of β -sheets (strong characteristic peaks at 1665.99 cm^{-1} , 1235.64 cm^{-1} , 1218.68 cm^{-1} , and 1086.51 cm^{-1}) and small amounts of α -helix (relatively weak characteristic peaks at 1267.32 cm^{-1} and 1101.78 cm^{-1}) and random coil (weak characteristic peaks at 1257.95 cm^{-1}). With the addition of PEG, the characteristic peaks at 1646.67 cm^{-1} and 1269.36 cm^{-1} attributed to α -helix disappear, whereas new characteristic peaks attributed to β -sheets (1241.65 cm^{-1} and 1078.45 cm^{-1})

FIGURE 3: X-ray diffraction curves of *A. pernyi* silk fibroin hydrogels (a) and *B. mori* silk fibroin hydrogels (b), the ratio of PEG was 0 (A), 100 wt% (B), and 300 wt% (C).

FIGURE 4: FT-IR spectra of *A. pernyi* silk fibroin hydrogels (a) and *B. mori* silk fibroin hydrogels (b), the ratio of PEG was 0 (A), 100 wt% (B), and 300 wt% (C).

appear in curve (B) of Figure 5(a). In Figure 5(b) curve (B), the characteristic peaks attributed to α -helix (1267.32 cm^{-1} and 1101.78 cm^{-1}) and random coil (1257.95 cm^{-1}) disappear with the addition of PEG, while those of the β -sheets (1666.99 cm^{-1} , 1240.30 cm^{-1} , 1086.51 cm^{-1} , 1064.22 cm^{-1} , and 1038.12 cm^{-1}) show in the curve.

These results indicate that the main secondary structure in *A. pernyi* and *B. mori* SF hydrogels are β -sheets. With the addition of polyalcohol, more β -sheets in the secondary structure of both *A. pernyi* and *B. mori* SF hydrogels were

formed. This is consistent with the results of X-ray diffraction and FT-IR.

Based on the above results and discussions, we would like to make some further points. Gelation occurs because SF chains tend to aggregate, passing from an amorphous (random coil) or unstable (α -helix) conformation to a more stable structure (β -sheets). The reactive activity of *A. pernyi* SF is stronger than that of *B. mori* SF due to its molecular structure. Therefore, the transition to β -sheets in both the pure *A. pernyi* SF aqueous solution and the solutions blended

FIGURE 5: Raman spectra of *A. pernyi* silk fibroin hydrogels (a) and *B. mori* silk fibroin hydrogels (b), the ratio of PEG was 0 (a), 300 wt% (b).

with PEG would be more rapid and much easier. Gelation of *B. mori* SF is both time- and energy-consuming, which has also been proven in other research [6, 7, 24]. In the present study, the gelation time of *A. pernyi* SF was proven to be much shorter than that of *B. mori* SF, even when nonauxiliary materials were added. Besides, adding even a small amount of PEG could reduce the gelation time. It has been proven that *A. pernyi* SF has better bioproperties than does *B. mori* SF. Therefore, *A. pernyi* SF hydrogels can be a more promising biomaterial.

The results of X-ray diffraction, FT-IR, and Raman spectroscopy indicate that the pure SF hydrogels (both *A. pernyi* and *B. mori*) contain mainly β -sheets, coexisting with a small amount of α -helix and random coil structures. With the addition of PEG, the amount of α -helix and random coil present is reduced, while more β -sheets are formed. This is probably because part of the α -helix and random coil structures have been transformed into β -sheets. The addition of PEG also reduces the gelation time, as described previously. This would further indicate that, while the addition of polyalcohol did not influence the main secondary structure of the hydrogels, it did induce a quicker transition to β -sheets and triggered additional formation of β -sheets.

4. Conclusions

Hydrogels from pure *A. pernyi* SF, pure *B. mori* SF, and SF blended with PEG were obtained and their secondary structures were studied. The conclusions of this study are as follows. (1) The gelation time of pure *A. pernyi* SF was significantly shorter than that of *B. mori* SF. (2) The gelation time of *A. pernyi* SF can be reduced even more by adding a small amount of PEG. (3) The addition of polyalcohol did not influence the main secondary structure of the hydrogels.

However, it did induce a quicker transition to β -sheets and triggered additional formation of β -sheets.

In this study, hydrogels from wild (*A. pernyi*) SF were successfully prepared in a much shorter time compared to hydrogels prepared from domestic (*B. mori*) SF. Considering their better bioproperties, *A. pernyi* SF hydrogels are a promising biomaterial that can be applied in much wider biofields.

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