Journal of Applied Polymer Science

Rapid formation of flexible silk fibroin gel-like films

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ABSTRACT: Flexible silk fibroin gel-like films with microporous morphology were prepared from *B. mori* silk fibroin fibers directly solubilized in formic acid/$CaCl_2$ solvent. These films were characterized by several analysis techniques to determine the structure and properties of films. The pore size of gel-like films can be adjusted through SF concentration and Ca ions concentration. The controllable pore size in gel-like films was grew from 3–5 μm to 100 μm under the increase of fibroin concentration from 1.0 wt % to 8.0 wt %. At the same time, the water content of silk fibroin gel-like film decreased from 83.5 ± 3.4% to 68.2 ± 2.6%. With increasing Ca ions contents from 2.0 wt % to 10.0 wt % in dissolution process, the pore size and water content of silk fibroin gel-like films grew larger, especially its water content values reached 86.2 ± 4.0% at 10.0 wt % Ca ions concentration. At wet condition, the gel-like film with β-sheet structure showed higher breaking stress (4.26 ± 0.31 MPa) and elongation (45.45 ± 15.79%) at 8.0 wt % concentration. With the preparation method, the membrane is hydrophilic and the pore size is adjustable, which contributes to high toughness and favorable cell growth environment, suggesting that these silk fibroin gel-like films can be a potential candidate scaffold for biomedical applications, such as wound dressing, facial mask, contact lenses, etc. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41842.

KEYWORDS: films; gels; morphology; porous materials; swelling

Received 27 September 2014; accepted 4 December 2014

DOI: 10.1002/app.41842

INTRODUCTION

Silk fibroin (SF), a fibrous protein, forms the filaments from the silk gland, which has been recognized as a potential new biomaterial.[1,2] Recently, many researchers have investigated SF as one of the most promising resources of biomedical materials, serving as bone regeneration, sustained drug delivery, and wound dressing, etc.[3–5] It can be manipulated into several material forms including films, three-dimensional porous scaffolds, nanofibers, and hydrogels. Before these formation process, the dissolution of SF is a very significant which is often required.[6–9]

SF molecule consists of heavy (H) chain of 390 kDa and light (L) chain of 25 kDa connected by a disulfide linkage. Its primary sequences of amino acid have been identified recently, consisting of highly repetitive Gly-Ala repeats.[10,11] In order to acquire regenerated silk for particular applications, alternative method of silk dissolution from cocoon is a challenging and meaningful work, which limited us to further formation.[12,13] In the traditional method, regenerated SF is achieved through a dissolution and coagulation process. Its regenerated process usually proceeds via following steps: the dissolution of native degummed silk in high salt solutions, such as 9.3 M LiBr solution or $CaCl_2$/Ethanol/H_2O solution (1: 2: 8 molar), followed by dialysis to remove the salt. Finally, various SF materials were acquired from regenerated SF solution via different post-treatments.[14,15] However, the mechanical properties of regenerated SF materials are generally poorer when compared with native silk fibers, which greatly limited their applications.[16,17]

SF hydrogels are of interest for many biomedical applications, and its formation process depends on the solution concentration, temperature, and pH, etc. Kim *et al.* studied the gelation parameters such as concentration, temperature, and pH, etc. affecting the gelation of SF solution.[18] Akira Matsumoto *et al.* elaborated the mechanism of SF sol−gel transitions.[19] In these researches, silk gelation time is relatively long unless nonphysiological treatments are considered (such as low pH, high temperature, and additives, etc.) in the absence of chemical modifications to silk protein.[20] At the same time, these hydrogels mainly exhibit leaflike and/or porous structure after lyophilization.

The dissolution of silk fibers using ionic liquids has been previously reported.[2,12,21] The advantage of ionic liquids is that it

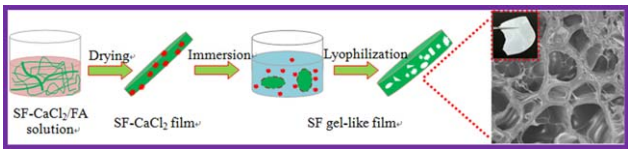

Figure 1. The schematic process of fibroin gel-like film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

simplified steps required for the dissolution process, as silk fiber can be dissolved directly into selected ionic liquids at room temperature. Therefore, we have been used ionic liquids (formic acid/$CaCl_2$, FA/$CaCl_2$) to prepare novel SF films.[21,22] This method dissolves silk without destroying the nanofibrillar structure, thus allowing flexibility and controllable in processing methodology with a simple operation and high dissolution efficiency. The prepared films with the main characteristic of gels such as high water content, nanofiber network morphology etc. were called gel-like films. Several analytical techniques were used to characterize its structure and properties.

EXPERIMENTAL

Materials

B. mori silk fibers were purchased from Zhejiang, China. Chemical regents (calcium chloride, sodium carbonate, formic acid, etc.) were bought from Sinopharm Chemical Reagent (Shanghai, China), and used without any further purification.

Preparation of SF Gel-Like Films

SF gel-like films were prepared as follows: *B. mori* silk fibers were boiled for 30 min in 0.05 wt % Na_2CO_3 solution at 100°C, rinsed thoroughly and dried. The fibroin fibers were dissolved in FA/$CaCl_2$ solvent, which SF content were 8.0 wt %, 6.0 wt %, 4.0 wt %, 2.0 wt %, and 1.0 wt %, respectively. Meanwhile, the concentration of $CaCl_2$ was ranged from 2.0 wt % to 10.0 wt %. After stirring 3 h at room temperature, SF solutions were cast on polystyrene dishes (diameter 90 mm) for drying, and then, these samples were immersed in deionized water to remove salt ions, obtaining gel-like films. The schematic process of fibroin gel-like film was shown in Figure 1. As control, SF films were prepared by traditional dissolution method.[23,24] Degummed SF fibers were dissolved in 9.3*M* LiBr solution at 70°C for 4 h. This solution was dialyzed against deionized water using dialysis tubes with 3500 kDa (Sigma, USA) for 72 h to remove the salt ions. After filtering, SF solution was cast on glass dishes and dried slowly to obtain films at room temperature.

Characterization

After lyophilization, the morphology of SF gel-like films was platinum-coated and examined by scanning electron microscope (SEM, Hitachi S4800, Japan). At the same time, the morphology of SF in dissolved process of FA/$CaCl_2$ solvent was also observed by SEM. To clearly characterize the dissolved process of silk fiber, silk fiber was dissolved in FA/$CaCl_2$ solvent with 4.0 wt % concentration at room temperature. In this dissolved solution, the concentration of $CaCl_2$ was 4.0 wt %. After stirring for 3 h, SF solution was diluted to below 0.001 wt % by FA solution and dropped on a silicon pellet surface with 1 μL. SF solution was quickly dried by liquid nitrogen at room temperature.

The secondary structure of SF films was analyzed by FTIR on Nicolet5700 (Thermo Nicolet Company, USA) in transmittance mode. For each measurement, each spectrum was obtained by the performance of 32 scans with the wavenumber ranging from 400 to 4000 cm^{-1}. At the same time, X-ray diffraction experiments were also measured on X Pert-Pro MPD (PANalytical, Netherlands) in transmittance mode to investigate the crystalline structure of samples. The incident beam wavelength was 0.154 nm. The intensity was finally corrected for changes in the incident beam intensity, sample absorption, and background.

SF gel-like films were immersed in distilled water at room temperature for 24 h. After excess water removing, the wet weight of the film (W_s) was measured. Samples were then dried in an oven at 100°C, and the dry weight of film (W_d) was also measured. The water content in the films was calculated as follows:[25]

$$\text{Water content } (\%) = \frac{W_s - W_d}{W_s} \times 100\,. \quad (1)$$

SF gel-like films at wet state were cut into 100 mm length with 100–200 μm thickness. The samples were tested by using automatic tensile tester (model3365, Instron, Boston, USA) to characterize the mechanical properties of films. During test process, distance between grips and test speeds were set to 20 mm and 10 mm min^{-1}, respectively. At the same time, the pre-tension was 0.2 cN. An average of 10 measurements was reported as the mean ± standard deviation for each sample. The formulas for breaking strength and extension at break were as follows:[2]

$$\text{Breaking strength } (\text{MPa}) = \frac{\text{Breaking force } (\text{N})}{\text{thickness } (\text{mm}) \times \text{width } (\text{mm})}; \quad (2)$$

$$\text{Extension at break } (\%) = \frac{\text{Specimen elongation } (\text{mm})}{\text{Original length } (\text{mm})} \times 100. \quad (3)$$

Statistical Analysis

All values were expressed as mean ± standard deviation. Statistical differences were determined by a Mann-Whitney *U* test (independent *t* test, SPSS).

RESULTS AND DISCUSSION

Preparation Process Analysis of Fibroin Gel-Like Films

In order to fabricate fibroin gel-like films, SF solution preparation is necessary and important. We employed FA/$CaCl_2$ solvent to dissolve degummed silk to prepare SF solution. We found that degummed silk was very soluble in FA/$CaCl_2$ solution at room temperature.[22] The use of FA/$CaCl_2$ solvent for silk dissolution was considered simple and efficient compared with the traditional solvents (9.3*M* LiBr or $CaCl_2$/Ethanol/H_2O solution). The dissolution behavior featured nanofibril-preservation (Figure 2), which was confirmed by SEM. The dissolution process of SF fibers in FA/$CaCl_2$ solution was assessed in Figure 2. Figure 2(a) shows images of natural fibroin fiber with 11 ± 2 μm. This natural fibroin fiber was obtained by degumming (0.05 wt % Na_2CO_3 solution), which not only removed sericin from silk fiber, but also weakened the noncovalent and covalent interactions, resulting in severe damage to the hierarchical structure of SF. When SF fiber was dissolved in FA/$CaCl_2$

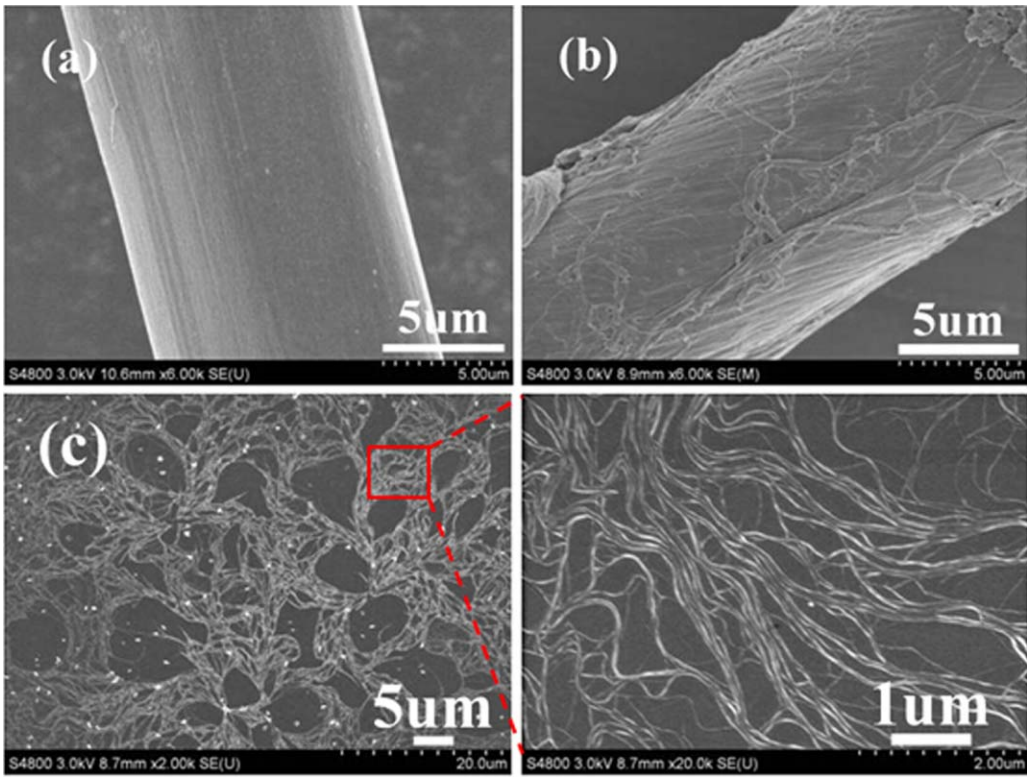

Figure 2. SEM images of SF fiber dissolution process in FA/$CaCl_2$ solvent: (a) Native SF fiber; (b) Well fibril structure in native silk; (c) After dissolution, nanosized silk fibrils were observed in FA/$CaCl_2$ solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

solution, well fibril structures were observed in native SF fiber [Figure 2(b)]. After completely dissolved, silk fiber was dissociated into nanofibrils with diameters from 20 to 200 nm in solutions [Figure 2(c)].

In addition, the formation of regenerated SF solution containing native fibrils may have useful application for preparing novel advanced materials. In this experiment, the 4.0 wt % SF nanofibril solution meant that 0.4 g native SF fiber dissolved in 9.6 g FA/$CaCl_2$ solution. In SF-FA-$CaCl_2$ solution, the proportion of SF and $CaCl_2$ was 1/1. After stirring 3 h at room temperature, SF solution was used to prepare gel-like films. The surface and cross-section of gel-like films showed porous structure (Figure 3).

Effect of SF Concentration on the Formation of Gel-Like Films

Multiple methods were used to dissolve silk fibers to prepare SF films with smooth surface. In these preparation processes, SF fibers were dissolved in a high-concentration, aqueous lithium salt solution or $CaCl_2$/ethanol/H_2O solution. These solutions were dialyzed to remove the salt, and had a lengthy process. However, this article presents an efficient process for preparing SF gel-like films with microporous morphology rather than reported SF compact films.[24] Figure 4(a–e) depicts the morphology of SF films prepared by FA/$CaCl_2$ solvent with different SF contents.

As control, SF film with smooth surface was prepared by traditional dissolution method [Figure 4(f)]. When SF gel-like films were prepared by FA/$CaCl_2$ solvent, many larger pores with 20–30 μm diameter were formed at 1.0 wt % concentration [Figure 4(a)], and the wall of holes were covered with a great deal of tiny holes (3–5 μm diameter). At the same time, the secondary structure of SF film was examined by FTIR. The absorption bands at 1647 cm^{-1} (Amide I) and 1540 cm^{-1} (Amide II) were attributed to random coil conformation [Figure 4(h)]. When SF concentration was increased to 2.0 wt %, the film exhibited porous structure with pore diameter 10–20 μm, not forming tiny holes on the wall [Figure 4(b)]. Moreover, larger microporous morphology with more than 50 μm diameter appeared on the SF films, when the concentration was increased from 2.0 wt % to 6.0 wt % [Figure 4(c,d)]. At 8.0 wt % concentration, many larger pores with more than 100 μm size were shown in Figure 4(e). At the same time, Figure 4(h) shows the absorption bands at 1631 and 1523 cm^{-1}, assigned to β-sheet structure. The microporous formation in gel-like films was attributed to ice crystallite formation in the freeze drying process. At the same time, the structural transformation from random coil to β-sheet was correlated to the dissolution of silk fiber in FA/$CaCl_2$ solvent.

The swelling degree of the fibroin films was measured based on the equilibrium swell ratio [Figure 4(g)]. When considering the possible applications in wounding dressings, etc., the ability of film to preserve water is one of the most important factors in the determination of the usefulness of biomaterials. The water content of the film prepared by FA/$CaCl_2$ solvent at 1.0 wt % concentration was 83.5 $\pm$ 3.4%, However, as control, the water content of fibroin film was only 36.1 $\pm$ 1.7%. The different is

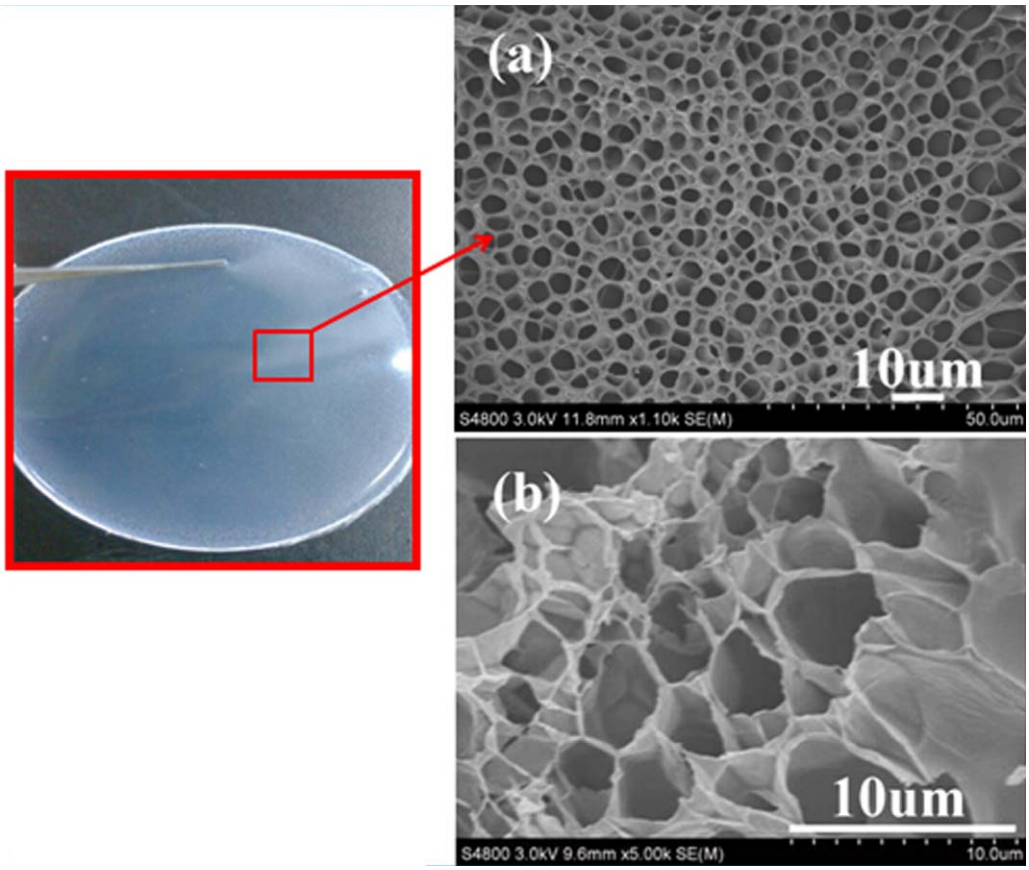

Figure 3. SEM micrographs of gel-like surface (a) and cross-section (b) (fracture in liquid nitrogen). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mainly attributed to the microporous morphology of SF gel-like films. When SF concentration was increased from 1.0 wt % to 6.0 wt %, the water content of films decreased from 83.5 ± 3.4% to 68.5 ± 2.8%. Even when the concentration was 8.0 wt %, the water content of film was also maintained at 68.2 ± 2.6%, higher than control samples. In fact, the water content of film not only relates to the porosity of SF films, but also associates with the structure of SF.[26]

Effect of Ca Ions Contents on the Formation of Gel-Like Films

In the preparation process, SF fibers were dissolved in FA/$CaCl_2$ solvent, and cast on polystyrene dishes for drying. And then, these samples were immersed in deionized water to remove salt ions, obtaining gel-like films. Hence, the morphology of SF gel-like films was also influenced by Ca ions contents in FA/$CaCl_2$ solvent. Figure 5 depicts SEM micrographs of SF gel-like films influenced by Ca ions contents. The concentration of Ca ions was 2.0 wt % in FA/$CaCl_2$ solvent, the morphology of SF gel-like films was not smooth, covering with a large number of micropores [Figure 5(a)]. At the same time, the secondary structure of films was examined by XRD [Figure 6(a)]. As comparison, SF film was treated with ethanol, exhibiting β-sheet structure [Figure 6(a–f)]. Compared with control sample, Figure 6(A-a) shows the diffraction peaks at 9.2° and 19.7°, exhibiting β-sheet structure. When the concentration of Ca ions was increased to 8.0 wt % in FA/$CaCl_2$ solvent, SF films exhibited porous structure with pore size 40–80 μm [Figure 5(b–d)]. Furthermore, the morphology of SF gel-like films, prepared at 10.0 wt % Ca ions concentration of FA/$CaCl_2$ solvent, showed larger pores with 60–80 μm [Figure 5(e)]. It is thus clear that the pore size rise following Ca ions concentration increase from 2.0 wt % to 10.0 wt %. At the same time, the prepared SF gel-like films showed similar secondary structure at different Ca ions concentration in dissolution process. The diffraction peaks at 9.2°, 20.6°, and 24.2° were seen, attributing to β-sheet structure [Figure 6(A-b,c,d,e)].

When SF gel-like films was prepared by FA/$CaCl_2$ solvent with 2.0 wt % Ca ions content, the water content was 63.4 ± 2.6%. The Ca ions content was increased from 2.0 wt % to 10.0 wt %, the water contents of films were 68.6 ± 3.1%, 74.4 ± 3.3%, 84.1 ± 2.9%, and 86.2 ± 4.0%, respectively. These results demonstrated SF gel-like films had higher water holding ability than pure SF films prepared by traditional dissolved method, such as 9.3*M* LiBr or $CaCl_2$/Ethanol/H_2O (1: 2: 8 molar).

Mechanical Properties of SF Gel-Like Films

The mechanical properties in the wet state are of primary importance for the determination of material performances expected to undergo various types of stresses.[26] In this study, the stress−strain curves were obtained in the wet state. According to literatures, the breaking stress of SF films at wet state was

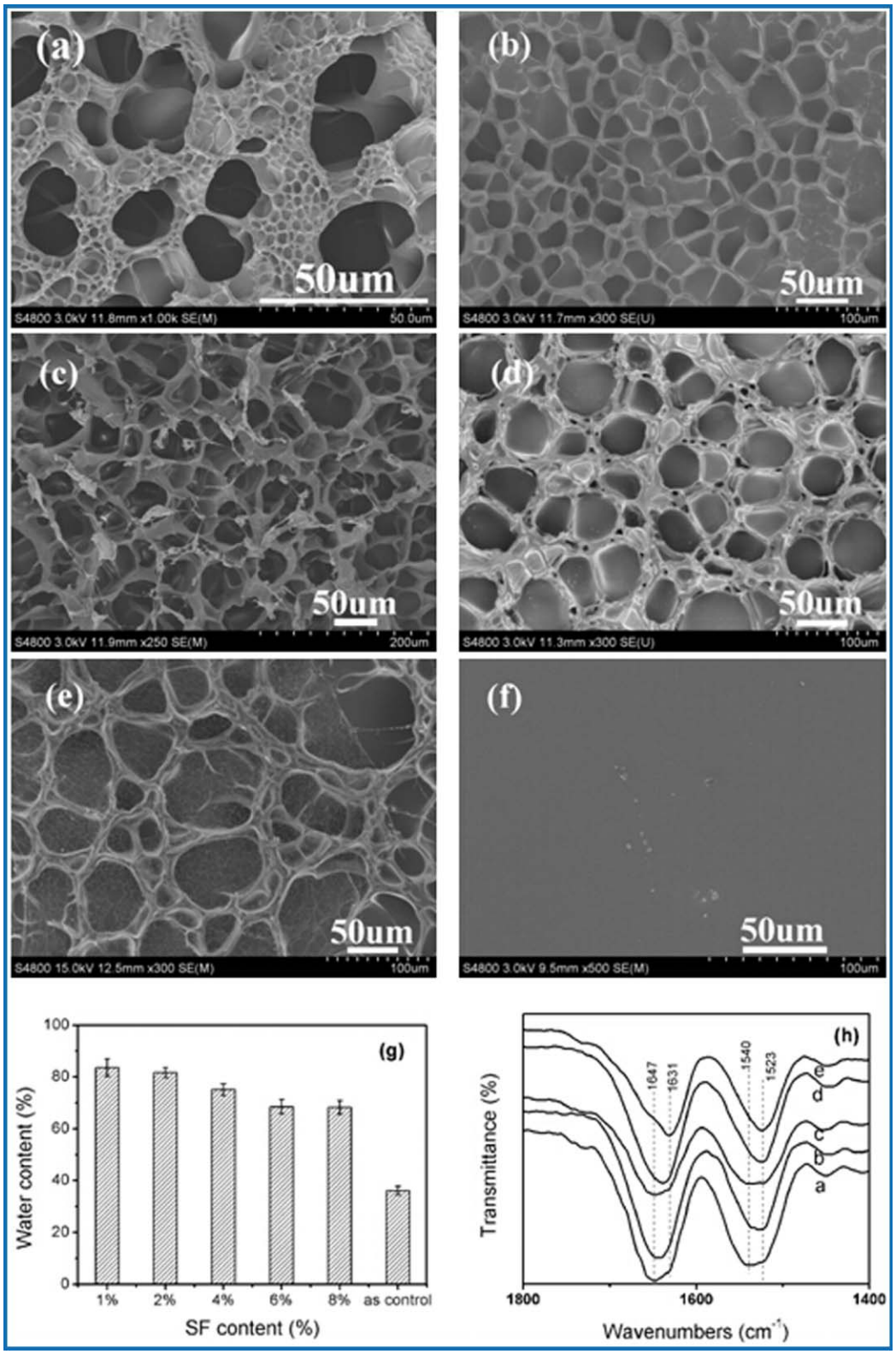

Figure 4. SF gel-like films prepared by FA/$CaCl_2$ solvent with different SF contents: (a) 1.0 wt %, (b) 2.0 wt %, (c) 4.0 wt %, (d) 6.0 wt %, and (e) 8.0 wt %, respectively; (f) the control sample; at the same time, water content (g) and FTIR spectra (h) of SF gel-like films. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

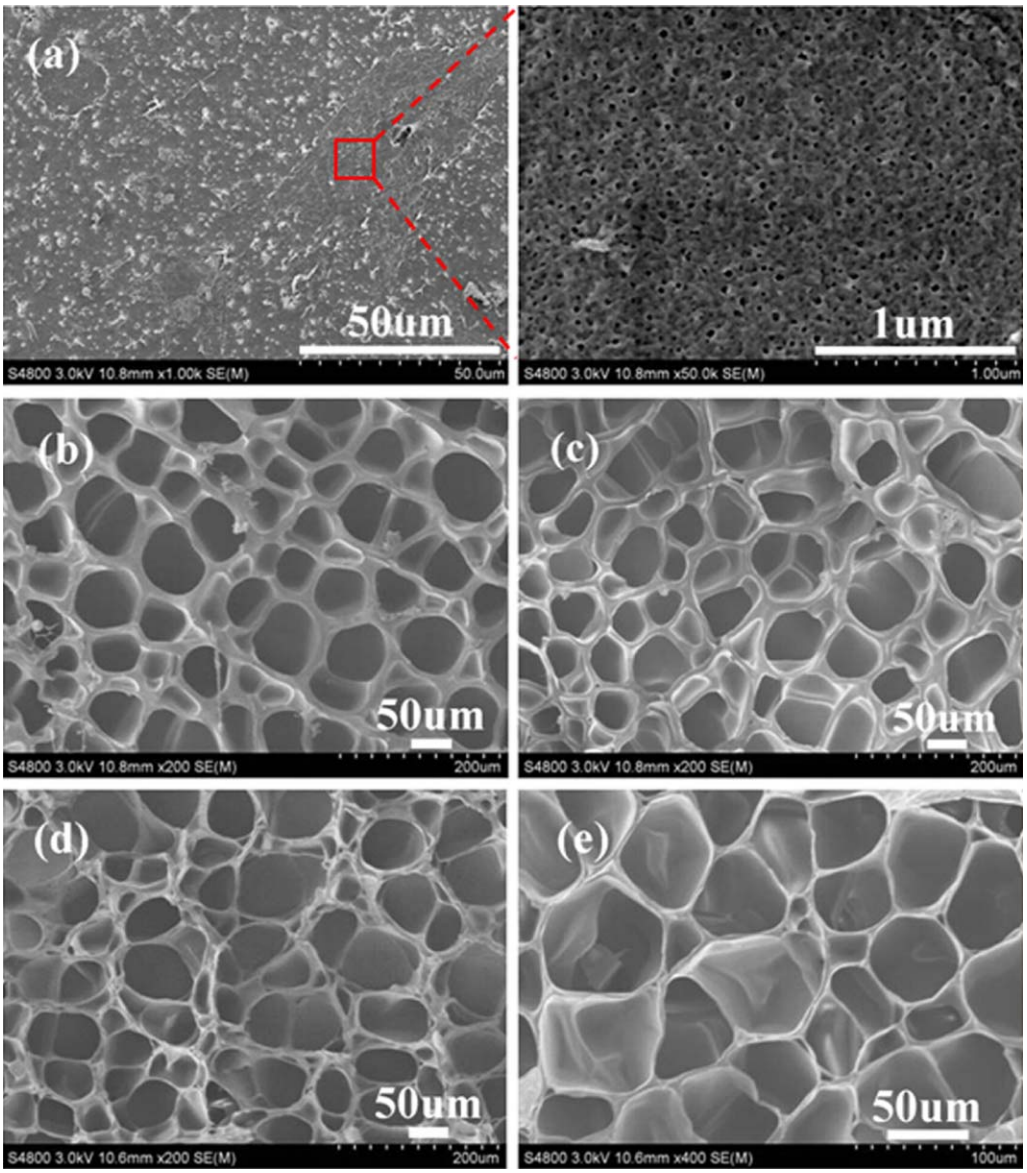

Figure 5. SEM micrographs of SF gel-like films influenced by ion contents. The ion contents were as follows: (a) 2.0 wt %, (b) 4.0 wt %, (c) 6.0 wt %, (d) 8.0 wt %, and (e) 10.0 wt %, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

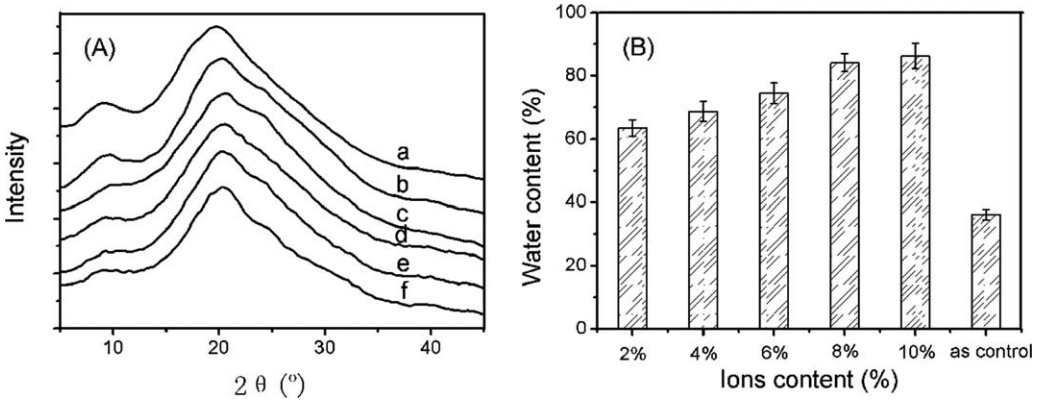

Figure 6. XRD (A) and water content (B) of SF gel-like films influenced by ion contents. The ion contents were as follows: (a) 2.0 wt %, (b) 4.0 wt %, (c) 6.0 wt %, (d) 8.0 wt %, (e) 10.0 wt %, and (f) as control, respectively.

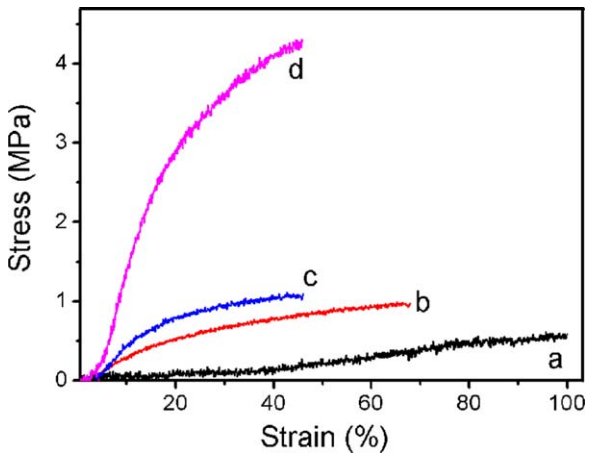

Figure 7. Mechanical properties of SF gel-like films prepared by FA/$CaCl_2$ solvent with different SF contents: (a) 2.0 wt %, (b) 4.0 wt %, (c) 6.0 wt %, and (d) 8.0 wt %, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

worse than that of dry state. The breaking stress and extension at break was 4.4 ± 0.6 MPa and $125 \pm 40\%$ for SF film, prepared by traditional dissolution method (9.3*M* LiBr) after methanol annealing at dry state.[27] However, SF gel-like films exhibited excellent flexibility at wet state. The results of the mechanical tests were shown in Figure 7. The breaking stress and extension at break were 0.56 ± 0.38 MPa and $115.49 \pm 25.8\%$, respectively, for SF gel-like film prepared at 2.0 wt % concentration [Figure 7(a)]. Figure 7 exhibits the breaking stress of SF gel-like film increased with the increase of SF concentration at wet condition. When SF films were prepared at 4.0 wt % and 6.0 wt % concentration, the breaking stress was 0.98 ± 0.25 MPa and 1.08 ± 0.27 MPa, respectively [Figure 7(b,c)]. In addition, at 8.0 wt %, SF gel-like film showed higher breaking stress and elongation at break for 4.26 ± 0.31 MPa, and $45.45 \pm 15.79\%$ [Figure 7(d)]. This phenomenon was mainly attributed to the β-sheet content of SF in films.[28] The secondary structure of SF gel-like films formed using different SF concentration was characterized by deconvolution of FTIR spectra [Figure 4(h)]. The relative ratios of different secondary conformations were calculated from the Amide I region. When SF concentration was from 1 wt % to 8 wt %, the β-sheet contents were $20.0 \pm 0.2\%$, $22.4 \pm 0.3\%$, $30.3 \pm 0.6\%$, $33.5 \pm 1.1\%$, and $40.8 \pm 2.1\%$, respectively. Following the increase in β-sheet content, the mechanical properties of SF gel-like films were increased.

CONCLUSIONS

This work presented a simple and efficient method for gel-like films made by *B. mori* silk fibroin fibers solubilized in FA/$CaCl_2$ solvent. The resulting SF gel-like films showed pore structure with diameter from 10 to 100 μm. With the fibroin concentration increasing from 1.0 wt % to 8.0 wt %, the pore size of SF gel-like films increased from 20 μm to 100 μm and its water content decreased from $83.5 \pm 3.4\%$ to $68.2 \pm 2.6\%$. However, following the increase of Ca ions contents in dissolved solution, the pore sizes also grew larger. At the same time, SF gel-like films had higher water holding ability, its values reaching $86.2 \pm 4.0\%$ at 10.0 wt % Ca ions concentration. Furthermore, at wet condition, SF gel-like films showed breaking stress and elongation at break for 4.26 ± 0.31 MPa and $45.45 \pm 15.79\%$ at 8.0 wt % SF concentration. Therefore, this article was provided a novel method to fabricate high-performance SF materials for biomedical applications, such as wound dressing, facial mask, contact lenses, etc.

ACKNOWLEDGMENTS

The authors are gratefully acknowledged the support of the Second Phase of Jiangsu Universities' Distinctive Discipline Development Program for Textile Science and Engineering of Soochow University, National Science Foundation of China (No. 81271723), and National Engineering Laboratory for Modern Silk. The Research and Innovation Project for College Graduates of Jiangsu Province (CXZZ13_0817).

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