Fabrication of Air-Stable and Conductive Silk Fibroin Gels
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Supporting Information

ABSTRACT: Owing to their promising applications in flexible electronics, researchers have extensively explored flexible and conductive gels. However, these gels have unsatisfactory strength and flexibility as well as easily dry in air. Herein, a rationally designed robust regenerated silk fibroin (RSF)-based gel with significant flexibility and strength, favorable conductivity, and excellent air stability is fabricated by inducing the conformation transition of RSF from random coil to β-sheet in ionic liquid (IL)/water mixtures. We found that such RSF-based gels have a unique homogeneous network structure of RSF nanofibers, which is likely formed because of evenly distributed cross-links dominated by small-sized β-sheet domains created during the conformation transition of RSF. Although the unique homogeneous nanostructure/network contributes toward improving the mechanical properties of these gels, it also provides pathways for ionic transport to help the gels preserve high conductivity of ILs. The prepared RSF-based gels display a remarkable air stability and reversible loss/absorption water capability in a wide humidity range environment primarily because of the distinguished combination of the IL and water. Therefore, the novel RSF-based gels hold a great potential in various applications as multifunctional, flexible, conductive materials, which are dispensed with encapsulation.

KEYWORDS: regenerated silk fibroin, flexible conductive gel, ionic liquid, β-sheet conformation, air stability

1. INTRODUCTION

In recent years, flexible conductive gels have gained an increasing popularity and been widely explored because of their promising applications in modern flexible electronics and mobile energy sources, such as smart sensors, flexible touch panels, and wearable energy devices.1–3 However, the traditional conductive gels based on conducting polymers, such as polyaniline and polypyrrole, display poor mechanical properties in strength and toughness, which limit their further applications.4 A few efforts have been made to fabricate flexible conductive gels through sequential self-assembly and a chemical cross-linking strategy5 or introducing silica nanoparticle cross-linkers in the polymer networks.6

However, almost all the gels easily dry and then harden in an air environment because the widely used solvents are volatile and gradually evaporate from the gels. The loss of solvent dramatically affects the properties of the gels. Therefore, such poor air stability of gels still limits their practical applications. Recently, a few kinds of air-stable and conductive gels have been prepared by the introduction of glycerol7 or ionic liquid (IL)/water mixtures.8,9 Among these, IL-incorporated conductive gels show great potential in modern flexible electronics because of their good air stability and mechanical properties.10 However, these air-stable, tough, and conductive gels are chemically cross-linked with the covalent network structures whose preparations require complex and multistep polymerization. Nevertheless, the chemically cross-linked network is stable but made irreversible.

As an abundant and sustainable natural protein harvested from Bombyx mori silkworm silk, regenerated silk fibroin (RSF) has been used to produce the silk protein-based materials with various shapes and properties, including films, hydrogels, particles, scaffolds, etc., for a wide range of applications.11–16

The traditional physically cross-linked silk fibroin hydrgel can be easily fabricated from the RSF aqueous solution by inducing the conformation transition of silk fibroin chains from random coil to β-sheet structures.17,18 However, most of these silk hydrogels show unsatisfactory mechanical performance because the “amphiphilic” silk fibroin chains tend to aggregate spontaneously in the RSF solution. In other words, it is easy for RSF to develop the large and unevenly distributed β-sheet aggregates to be the cross-linker of the RSF hydrgel, resulting in poor mechanical properties. Although construction of a homogeneous silk network with uniformly dispersed cross-linking points (β-sheet structures) via chemical19 and physical20,21 methods has been one of the potential strategies to improve the mechanical properties of the RSF hydrgel, the robust silk hydrogels still lack other properties such as conductivity and long-time air stability.

In general, the ILs can well dissolve the degummed silk fiber,22 and the RSF chains display the random coil conformation in the solution.23 An acetate IL, 1-ethyl-3-
methylimidazolium acetate (EMImAc), was particularly reported as a good solvent for RSF, and a sol–gel transition of the RSF/EMImAc/water system was observed under appropriate conditions. This provides us with an opportunity to fabricate a physically cross-linked RSF gel by inducing the conformation transition of RSF with water from a RSF/IL solution. On the basis of the investigation and understanding of the structure and properties of silk fibroin-based materials, in this work, we have developed a novel physically cross-linked RSF-based gel in order to combine well-performed mechanical properties, remarkable conductivity, and long-term air stability. All these properties were improved via evenly distributed cross-links dominated by small-sized $\beta$-sheet domains formed during the conformation transition of RSF, the conductive and nonvolatile IL, as well as its great moisture taking ability.

2. EXPERIMENTAL SECTION

2.1. Materials. The Bombyx mori silkworm cocoon was purchased from Shandong Province, China. EMImAc and other chemicals were purchased from the Lanzhou Institute of Chemical Physics (Lanzhou, China) and Aldrich, respectively, and were used as received.

2.2. Preparation of the RSF/EMImAc Solution. The degumming process of silk cocoons was carried out following a conventional procedure as described in previous work. In short, an aqueous solution of 0.5% NaHCO$_3$ was used to boil the cocoon (30 min, twice) to remove the sericin, and the obtained silk fiber was rinsed with deionized water and then dried at 40°C C under stirring for 2–4 h to ensure complete dissolution. The resultant RSF/EMImAc solutions with a series of concentrations (from 10 to 20 wt %) were hermetically stored in a desiccator to prevent moisture absorption.

2.3. Preparation of Different RSF-Based Gels. RSF/EMImAc/H$_2$O and RSF/EMImAc/EtOH–H$_2$O gels have the same preparation process except for the addition of denaturants, that is, deionized water for the RSF/EMImAc/H$_2$O gel and ethanol–water mixed solution (EtOH–H$_2$O) for the RSF/EMImAc/EtOH–H$_2$O gel. The procedure is as follows: deionized water or ethanol–water mixed solution (EtOH–H$_2$O) with different ethanol concentrations was slowly added into the RSF/EMImAc solution under gentle stirring to form a uniform RSF mixing solution. Before gelation, the RSF solution was centrifuged to remove the bubbles. All the samples with various RSF contents and denaturants were prepared and incubated at room temperature, and the gelation time was determined by an inverted test tube method.

The RSF/EMImAc/H$_2$O and RSF/EMImAc/EtOH–H$_2$O gels were marked as $x/y/1_z$, where $x$, $y$, and $z$ represent the content of RSF, EMImAc, and denaturant, respectively, and $z$ represents the concentration of ethanol in ethanol–water mixed solution. The RSF/EMImAc/H$_2$O gels can be designated as $x/y/1_{0.0}$ or be abbreviated as $x/y$.

To differentiate these naturally formed RSF hydrogels, the named RSF(I)-hydrogels were prepared from RSF/EMImAc/H$_2$O or RSF/EMImAc/EtOH–H$_2$O gel by immersing them in deionized water for complete removal of EMImAc and EtOH.

2.4. Measurement of Mechanical Properties. The mechanical testing of all RSF-based gels was carried out on an Instron 5565 universal testing machine (Instron, UK) at 25 ± 5°C and 50 ± 5% relative humidity (RH) with a load cell of 500 N. Compression tests were performed via a uniaxial compression test method. Cylindrical samples with a diameter of 12 mm and a height of 10 mm were fabricated, and the compression rate was set at 30%/min. For tensile tests, the samples were cut into a rectangular shape of 50 mm in length, 3 mm in width, and 1 mm in thickness. The gauge distance was 10 mm, and the cross-head speed was set at 10 mm/min.

2.5. Raman Spectroscopy. Samples of EMImAc, RSF/EMImAc solution, and RSF/EMImAc/H$_2$O gels were prepared. The Raman spectra of different samples were recorded with an Xplora Raman spectrometer (HORIBA Jobin Yvon, France) at an excitation wavelength of 532 nm.

2.6. Fourier Transform Infrared Spectroscopy (FTIR). RSF(I)-hydrogels were freeze-dried, powdered, mixed with KBr, and processed into pellets. The FTIR spectra were obtained with a Nicolet 6700 spectrometer (ThermoFisher, USA) in the range of 650–4000 cm$^{-1}$ with a resolution of 2 cm$^{-1}$ for 128 scans.

2.7. Field-Emission Scanning Electron Microscopy (FESEM) and Cryo-Field-Emission SEM (Cryo-SEM) Analysis. The morphology of the hydrogel was observed by a Hitachi S-4800 scanning electron microscope and a cryo-scanning electron micro-

Figure 1. (a) Sol–gel transition, (b) digital photos, and (c) cyclic compressive test of the RSF/EMImAc/H$_2$O gel ($[\text{RSF}] = 10$ wt %, $[\text{EMImAc}] = 40$ wt %, $[\text{H}_2\text{O}] = 50$ wt %). (d) Representative stress–strain compressive and (e) tensile curves of RSF/EMImAc/H$_2$O gels with different RSF contents and water contents as noted. All mechanical tests were performed at 25 ± 5°C with 50 ± 5% RH.
Sample of the naturally formed RSF hydrogel and the RSF(1)-hydrogel with the RSF content of 5 wt % were cut into pieces of 1 mm in thickness, quenched in liquid nitrogen for 5 min, and then freeze-dried. The lyophilized RSF hydrogel samples were coated with gold using a sputter coater (Hitachi e-1030) for 30 s before the FESEM observation. For cryo-SEM observations, both samples were loaded on the cryo-specimen holder and frozen rapidly by putting them into a liquid nitrogen bath, and then the liquid nitrogen bath was transferred into the vacuum space for an immediate vacuum. The samples were observed under a Hitachi S-4800 emission scanning electron microscope at low temperatures maintained by liquid nitrogen.

### 2.8. Synchrotron Radiation Small-Angle X-ray Scattering (SAXS) Analysis

Synchrotron radiation SAXS experiments were performed on the BL16B beamline of the Shanghai Synchrotron Radiation Facility (SSRF), Shanghai, China. The distance from the sample to detector was 1805 mm, and the X-ray wavelength was λ = 0.124 nm. The two-dimensional SAXS data were converted to one-dimensional intensity I(q) as a function of the scattering vector q [q = (4π/λ)sin θ] by circular averaging, where 2θ is the scattering angle. All the gel samples with the cut dimension of 10 × 10 × 1 mm³ were used for SAXS analysis.

### 2.9. Thermogravimetric Analysis (TGA)

Thermal degradation of different RSF-based gels was performed on a thermogravimetric analyzer (Pyris 1, PerkinElmer, USA) from 20 to 400 °C at a speed of 10 °C/min under nitrogen atmosphere.

### 2.10. Conductivity Properties

The impedance measurements of RSF-based gels were performed using an electrochemical workstation (CHI660e), where the gel sample was pasted between two symmetrical conductive silver adhesives. All the gels were cut in a rectangular shape with measurements of 25 ± 0.5 mm in length, 3 ± 0.5 mm in width, and 2 ± 0.5 mm in thickness, and the impedance measurements were performed in an ambient atmosphere at room temperature (25 ± 5 °C).

### 3. RESULTS AND DISCUSSION

#### 3.1. Preparation and Mechanical Properties of RSF-Based Gels

At first, we used deionized water as a denaturant to prepare the RSF/EMImAc/H₂O gel, according to the procedure described in the Experimental Section. The RSF/EMImAc/H₂O solution (left image in Figure 1a) gradually changed into white or faint yellow gel (right image in Figure 1a) after a period of time at room temperature. The gelation time or gelation process is influenced by the temperature, RSF content, water content, and other factors. For example, when RSF, EMImAc, and H₂O were 10, 40, and 50 wt %, respectively, the mixture solution took about 9 days to form a gel at the room temperature (Figure 1a). In contrast with the pasty RSF hydrgels naturally formed from the RSF aqueous solution, the RSF/EMImAc/H₂O gel displayed improved mechanical performance and good stability to bear different external forces including large bending or twisting (Figure 1b), which is directly correlated with the flexibility and elasticity of the RSF/EMImAc/H₂O gel.

The mechanical properties of various RSF/EMImAc/H₂O gels were quantitatively investigated through compression and tensile tests, and the representative stress-strain curves as well as the loading-unloading cycles are shown in Figure 1c–e. In general, the RSF/EMImAc/H₂O gel withstood a strain as large as 20% and recovered quickly with a relatively small hysteresis cycle in the following repeated compression tests, indicating a good elasticity (Figure 1c).

#### Table 1. Mechanical Properties of RSF/EMImAc/H₂O Gels (n = 5 for Compression Tests and n = 20 for Tensile Tests)

<table>
<thead>
<tr>
<th>RSF/EMImAc/H₂O (wt %)</th>
<th>Compression modulus [MPa]</th>
<th>Compression stress at 60% strain [MPa]</th>
<th>Tensile modulus [MPa]</th>
<th>Breaking elongation [%]</th>
<th>Breaking stress [MPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/45/50</td>
<td>0.77 ± 0.09</td>
<td>0.35 ± 0.01</td>
<td>0.85 ± 0.11</td>
<td>51 ± 8</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>7.5/42.5/50</td>
<td>1.7 ± 0.2</td>
<td>0.75 ± 0.01</td>
<td>1.8 ± 0.1</td>
<td>90 ± 16</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>10/40/50</td>
<td>3.5 ± 0.1</td>
<td>1.06 ± 0.02</td>
<td>3.1 ± 0.3</td>
<td>94 ± 10</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>10/50/40</td>
<td>3.7 ± 0.3</td>
<td>1.22 ± 0.03</td>
<td>3.1 ± 0.3</td>
<td>147 ± 29</td>
<td>0.41 ± 0.02</td>
</tr>
</tbody>
</table>

#### 3.2. Thermal Degradation and Mechanical Properties of RSF/EMImAc/EtOH Gels

As compared to their counterparts, the mechanical performance of RSF/EMImAc/EtOH–H₂O gels with different ethanol concentrations ([RSF] = 10 wt %) showed an enhancement of the mechanical properties. For instance, when the RSF content increased from 5 to 10 wt %, the compressive stress of the gel increased from 0.35 ± 0.01 to 1.22 ± 0.03 MPa and the corresponding compression modulus increased from 0.77 ± 0.09 to 3.66 ± 0.27 MPa (curve A vs curve D in Figure 1d). In addition, the RSF/EMImAc/H₂O gel with little water content exhibited high compressive strength and compression modulus (curve D vs curve C in Figure 1d), suggesting that the proportion of water and IL could also affect the mechanical properties of RSF/EMImAc/H₂O gels with the same RSF content.

Generally, the natural polymer-based hydrogels consisting of a physically cross-linked network have poor mechanical properties, which could only be subjected to compression but were susceptible to stretching. However, the RSF/EMImAc/H₂O gels were strong enough to prepare specimens for tensile tests even when the RSF content was as low as 5 wt %. This is a qualitative leap compared to those naturally formed RSF hydrgels, which were normally too weak to run the mechanical tests. As shown in Figure 1c, the RSF/EMImAc/H₂O gels exhibited superior tensile mechanical performance; when the RSF content increased from 5 to 10 wt %, the corresponding mechanical properties of the gels increased several folds. Among these, the RSF/EMImAc/H₂O gel with the RSF content of 10 wt % and the water content of 40% showed the strongest mechanical performance. The detailed mechanical properties (including both compression and tensile data) are presented in Table 1.

Although the RSF/EMImAc/H₂O gel had remarkable mechanical properties, it might suffer from the long gelation time which varied from 30 days ([RSF] = 5 wt %) to 9 days ([RSF] = 10 wt %). It is well-known that alcohol can dramatically promote the conformation transition of silk fibroin from random coil to β-sheets and then accelerate the gelation process of RSF. Therefore, the ethanol–water mixed solution (EtOH–H₂O) with different ethanol concentrations was employed as an alternate of deionized water to prepare RSF/EMImAc/EtOH–H₂O gels. As a result, the gelation time of RSF-based gels was greatly reduced from days to a few hours, which could easily be tuned via variation of ethanol concentration (Figure S1).
structures in RSF/EMImAc/EtOH–H2O gels when ethanol with different concentrations was added, which is discussed later in the paper.

A robust RSF(I)-hydrogel could be obtained by immersing the RSF/EMImAc/H2O or RSF/EMImAc/EtOH–H2O gel in deionized water to remove all EMImAc and EtOH. The mechanical properties of RSF(I)-hydrogels were similar to those of their precursors (Figure S4 and Table S2), which are much better than those of the traditionally formed RSF hydrogels whose compressive modulus or tensile modulus was in the range of 0.01–0.1 MPa.26–28 For example, at a RSF content of about 10 wt %, the prepared RSF(I)-hydrogel exhibited superior mechanical performances under both compression and tensile mode, as its compression modulus reached more than 4.0 MPa, whereas the tensile modulus, strength, and breaking elongation reached up to 0.37 ± 0.04 MPa, 124 ± 36%, and 3.36 ± 0.23 MPa, respectively. However, there was a slight decrease in strength and elongation. These results suggest that the network of the RSF(I)-hydrogel may have inherited a similar structure as the RSF/EMImAc/EtOH–H2O gel, which is completely different from that of the naturally formed RSF hydrogel.

3.2. Structure and Morphology of RSF-Based Gels. In the current work, the sealed RSF/EMImAc solution was stable at the room temperature for more than 1.5 years without any precipitation or gel formation. However, once sufficient water was added into the RSF/EMImAc solution, the RSF/EMImAc/H2O mixture gelled with course of time (as specified above). Such a gelation process was induced by the water in which the RSF chains could spontaneously aggregate into β-sheets to form cross-links of the network. To characterize the conformation transition of RSF molecules during gelation, we collected the Raman spectra of the RSF/EMImAc/H2O mixtures at different incubation stages (Figure 2a). The amide I band of RSF (around 1600–1700 cm⁻¹) was detected for two reasons. First, the amide I band of protein was usually used to analyze its secondary structure,30,31 and the amide I bands around 1660 and 1682 cm⁻¹ have been reported to be associated with the β-sheet and random coil conformations of RSF, respectively.27 Also, there was no band around 1600–2000 cm⁻¹ in the Raman spectrum of EMImAc (Figure S5). As can be seen in Figure 2a, the Raman spectrum of the RSF/EMImAc solution showed a broad peak at amide I band around 1680 cm⁻¹ (curve A), indicating a predominant random coil conformation of silk fibroin. After deionized water was added to the RSF/EMImAc solution, the amide I peak of RSF in RSF/EMImAc/H2O mixtures gradually shifted to low wavenumber and narrowed as the time progressed (Figure 2a, curve B–curve D). These results reveal that the RSF conformation changed from random coil to β-sheet24 during the gelation process. Once the gel is formed, a clear sharp amide I band can be seen at 1662 cm⁻¹. The structure of the lyophilized RSF(I)-hydrogel was also characterized using FTIR (Figure 2b). The peaks at 1624 cm⁻¹ in the amide I region, 1515 cm⁻¹ in the amide II region, and 1229 cm⁻¹ in the amide III region appeared, indicating the domination of β-sheet conformation in the sample.32

SAXS is a vital tool to investigate the structure of mass fractal aggregates because it can probe the structure over several orders in length scale, including both the primary particle and fractal aggregates.33 Recently, synchrotron radiation SAXS was used to study the structure of hydrogels.34–36 To further understand the influence of ethanol on the microstructure of the gels, SAXS was employed to probe the microstructure of the RSF/EMImAc/H2O and RSF/EMImAc/EtOH–H2O gels. The RSF/EMImAc/EtOH–H2O gel with 40% (v/v) ethanol concentration showed a slight difference in mechanical performance as compared to that of the RSF/EMImAc/H2O gel (0% ethanol concentration).

Figure 3a displays the SAXS profiles of RSF/EMImAc/H2O gel and RSF/EMImAc/EtOH–H2O gel samples. There were no noticeable scattering peaks in I(q) versus q scattering data plotted on log–log scales, but a clear power-law behavior (I(q) ∝ q⁻p) was observed over two different length scales. The power-law exponents at low-q range and high-q range are shown in Figure 3b,c, respectively. A power-law scattering behavior is often suggestive of scattering from fractal structures, which is either the mass or the surface fractals. The fractal dimensions (Dm for the mass fractal dimension and Ds for the surface fractal dimension) are related to the power-law exponent p.34,39,40 For mass fractal, the Dm is equal to the power-law exponent p, which changes from 1 for loosely connected aggregates possessing indistinct interfaces to 3 for dense aggregates.34,40 Ds for surface fractals is equal to 6 − p, 3 for rough surfaces, and 2 for smooth surfaces.34,39,40

As can be seen in Figure 3b, at low-q range, the scattering spectra of two samples showed I(q) ∝ q⁻2 dependency of scattering intensity, whereas Dm was about 2.0 for the aggregates of both samples, indicating the presence of a low-dimensional network structure aggregated from primary particles in the gels. The diameter of the primary particles can be calculated from the crossover region in the scattering profiles [I(q) vs q scattering data plotted on log–log scales].34,35 According to Figure 3c, at high-q range, the RSF/EMImAc/H2O gel displayed a power-law exponent (p) of 3.9 which yielded Ds = 2.1, implying a smooth surface fractal morphology for the primary particles. While the RSF/EMImAc/EtOH–H2O gel had a surface fractal dimension Ds = 3.0 for the primary particles, which indicates a relatively rough surface.34,39,40

The expression of the intensity function in the form of the Kratky plots (q²I(q) vs q) has been reported to be useful for estimating the protein molecule folding situation in particles.42,43 In the case of protein folding, a clear peak in the Kratky plots indicates a compact globular structure, while the
peak position depends on the radius of gyration, $R_g$.\textsuperscript{35,38,42} As can be seen in Figure 3d, clear peaks in the Kratky plots for both RSF/EMImAc/H$_2$O gel and RSF/EMImAc/EtOH–H$_2$O gel were found, suggesting that the RSF in both gels had a globular shape with a folded structure or partially folded structure containing some random chains. The peak position changed to higher $q$ when the ethanol concentration increased from 0 to 40%, indicating that the RSF/EMImAc/EtOH–H$_2$O gel had a smaller $R_g$ value than that of the RSF/EMImAc/H$_2$O gel, which is consistent with the trend observed from the crossover region discussed above.

It is well-accepted that the characteristics of polymer gels are strongly related to their internal structure.\textsuperscript{44} The prepared RSF(I)-hydrogels showed outstanding mechanical properties that are superior to those of naturally formed RSF hydrogels, which motivated us to investigate their internal morphology/structure using FESEM and cryo-SEM analysis.

The SEM images of two lyophilized RSF hydrogels differed a lot from each other (Figure 4). As shown in Figure 4a, the cross section of the naturally formed RSF hydrogel consists of several large collapsed fragments or bricks of silk protein, which may be related to the large RSF aggregates caused by the pressure of ice crystals, whereas the RSF(I)-hydrogel had a relatively homogeneous network structure (Figure 4b) with clearly visible fibrils of about 16 nm in diameter that could be found randomly distributed all around (Figure 4c). The cryo-SEM observation of both samples provided more information in detail and showed clearer fibril structures. According to
cross-linking points dominated by smaller Such a unique structure was derived from uniformly dispersed constructed by large amounts of RSF nano morphology, where a homogeneous network structure was residue change of the RSF/EMImAc/H2O gel and RSF(I)-hydrogel during the as-prepared gels exposed to air (RT, 30 years). As alcohol could accelerate the gelation process and is not a necessary factor for the air stability of the RSF/EMImAc/EtOH–H2O gel, hereby, we only focused on the RSF/EMImAc/H2O gel and investigated its air stability in detail.

As shown in Figure 5a, the RSF/EMImAc/H2O gel with 40 wt % EMImAc and 50 wt % water almost maintained its original size and shape after being exposed to air (RT, 40–60% RH) for 20 days, whereas the RSF(I)-hydrogel shrunk gradually and dried out within 1 day. The RSF/EMImAc/H2O gel lost weight quickly over the first few hours after being exposed to air and then reached to a “balance” with increased exposure time. As confirmed gravimetrically, exposure of a RSF/EMImAc/H2O gel to air (RT, 30–60% RH) resulted in about 20% decrease in weight within 6 h, about 28% within 24 h, and then the weight varied within a small range during the following 20 days (curve A in Figure 5b). We proposed that the as-prepared RSF/EMImAc/H2O gel which contained amounts of “free water” (the water content was preset) would not be stable enough. Thus, after being exposed to air, the “free water” in gel evaporates, similar to other hydrogels. The nonvolatile and water-miscible IL we chose had a strong interaction with H2O to prevent its further escape in a certain environment. In other words, IL and H2O reached a delicate balance to preserve the network of the gel therein. However, the RSF(I)-hydrogel continuously lost all contained water within the initial 2 days of exposure, and its weight did not change during the following 18 days (curve A′ in Figure 5b). Considering the nonvolatility of IL, we proposed that the RSF/EMImAc/H2O gel lost its weight only because of the water evaporation, the water content retained in the gel could be principally calculated by the weight loss ratio (or water loss ratio), which was confirmed by the TGA experiment (Figure S6).

After being exposed to air for 20 days, the RSF/EMImAc/H2O gel consistently displayed a good mechanical performance, though it became more tough and soft as compared to the original gel (Figure 5c). The phenomenon could be

**Figure 5.** (a) Photographs of the RSF/EMImAc/H2O gel ([RSF] = 10 wt %, [EMImAc] = 40 wt %, [H2O] = 50 wt %) and the RSF(I)-hydrogel prepared from the RSF/EMImAc/H2O gel when exposed to air (RT, 40–60% RH) for different times (0 h, 12 h, 1 day, and 20 days). (b) Weight residue change of the RSF/EMImAc/H2O gel and RSF(I)-hydrogel during the as-prepared gels exposed to air (RT, 30–60% RH). The weight residue was \( W_t/W_0 \), where \( W_0 \) is the initial weight and \( W_t \) is the weight at different times. (c) Representative stress–strain compression curves (n = 5) of the as-prepared RSF/EMImAc/H2O gel and evaporated RSF/EMImAc/H2O gel after being exposed to air for 20 days (cyclic compressive tests of the evaporated RSF/EMImAc/H2O gel is inserted).
attributed to the virtual RSF content in the exposed gel, which was higher than that of the as-prepared one, as well as the increased EMImAc/H2O proportion which kept the network of the gel together even as the cross-linker of $\beta$-sheet tended to dissolve in IL. Moreover, the enhanced elasticity of the exposed RSF/EMImAc/H2O gel with small hysteresis cycles was confirmed (inset of Figure 5c).

The air stability of RSF/EMImAc/H2O gels was affected by the RH of the environment because of the binding between EMImAc and H2O. As shown in Figure 6a, after being exposed to an environment with a constant RH ranging from 75 to 7% for 3 days, the RSF/EMImAc/H2O gels changed little in shape and color as compared to the initial one. Regarding their mechanical performance, the compression modulus of RSF/EMImAc/H2O gels decreased and the elasticity increased with the decrease in RH. The details of these changes are displayed in Figure 5d of the Supporting Information. The air stability of RSF/EMImAc/H2O gels was confirmed (inset of Figure 5c).

Figure 6. (a) Photographs of the as-prepared RSF/EMImAc/H2O gel (noted as initial, [RSF] = 10 wt %, [EMImAc] = 40 wt %, [H$_2$O] = 50 wt %) and different evaporated RSF/EMImAc/H$_2$O gels exposed to the environment with a constant RH of 75, 57, 44, 23, and 7%, respectively, for 3 days (named 75RH-3, 57RH-3, 44RH-3, 23RH-3, and 7RH-3). (b) Representative stress-strain compression and (c) tensile curves of the as-prepared RSF/EMImAc/H$_2$O gel and the one of 7RH-3. The cyclic compressive tests of 7RH-3 are inserted in (b). All tests were performed at 25 ± 5 °C with 50 ± 5% RH ($n$ = 5 for compression tests and $n$ = 20 for tensile tests). (d) Weight residue changes of the as-prepared RSF/EMImAc/H$_2$O gel (curves of A–E) and RSF(I)-hydrogel (curves of A′–E′) during their exposure to the environment with a constant RH noted. The weight residue is $W_t/W_0$, where $W_0$ and $W_t$ are the original weight and the weight at a certain time. (e) Weight residue changes of the evaporated RSF/EMImAc/H$_2$O gels with different weight-loss ratios during their exposure to the environment with 75% RH (black curves of A, B, C) or 57% RH (red curves of D, E, F). The evaporated RSF/EMImAc/H$_2$O gels were prepared from the as-prepared RSF/EMImAc/H$_2$O gels after being placed to the environment with 23% RH for losing weight/water to various degrees. The weight residue of the as-prepared RSF/EMImAc/H$_2$O gels was set to 100%.
weight residue decreased with decreasing RH (curve B–curve E in Figure 6d). When exposed to an environment with a constant RH ranging from 75 to 7% for more than 2 days, the RSF/EMImAc/H2O gels had little change in weight and it seemed to reach a balance or stable state. In the case of controls, RSF(1)-hydrogels showed continuous weight loss apparently within 1 or 2 days of exposure in all different RH environments until the RSF(1)-hydrogel dried out and there was no more water to be lost (curve A'–curve E' in Figure 6d). The RSF/EMImAc/EtOH–H2O gel showed a trend similar to that of the RSF/EMImAc/H2O gel (Figure S8). Overall, it could be concluded that the RSF/EMImAc/H2O gels would not dry out and exhibited novel air stability when exposed to environments with a wide RH range.

We found that when RSF/EMImAc/H2O gels were kept in environments with different humidities, they exhibited reversible losing/adding weight process (losing/absorbing water process), which benefited from the extremely low vapor pressure and water-absorbing capacity of EMImAc. For instance, the as-prepared RSF/EMImAc/H2O gels were placed in a relatively dry environment with 23% RH for various times to obtain evaporated gels with different weight-loss ratios (losing weight process). After these “weight-loss” RSF/EMImAc/H2O gels were transferred to an environment with 75 or 57% RH, the weight of the gels gradually increased with increasing time by moisture absorption, as shown in Figure 6e. Moreover, when the RSF/EMImAc/H2O gels with different weight-loss ratios tended to increase to a similar weight residue after a few hours of exposure in a certain RH, the resulting weight residue increased to about 94% in environment with 75% RH (curve A–curve C in Figure 6e) and about 81% in 57% RH (curve D–curve F in Figure 6e). The resulting weight residue during the adding weight process was consistent with that during the losing weight process discussed above (Figure 6d), which further confirmed that the resulting weight residue of RSF/EMImAc/H2O gels was related to the environmental RH. The evaporated RSF/EMImAc/H2O gels with the weight residue as low as 58% (after exposure to the environment with 7% RH for 3 days) had limited water absorption ability, which is likely because the internal structure of gels would change if too much water was lost. To conclude, in contrast with the traditional RSF hydrogels, the RSF/EMImAc/H2O gel had some capability for reversible water loss/absorption of water, and the environmental RH played a critical role in achieving the final degree of water loss.

3.4. Conductivity of the RSF-Based Gels. In contrast with the RSF(1)-hydrogel, the RSF/EMImAc/H2O gel can act as a conductor to successfully connect a light-emitting diode (LED) circuit, exhibiting favorable conductivity (Figure S9 and Video S2 of the Supporting Information). We ascribed such properties to the high ionic conductivity of EMImAc and the unique nanostructure in gels. On one hand, the EMImAc in gel which served as the liquid electrolyte had appropriate ionic conductivity and more EMImAc content contributed to increasing the ionic conductivity of gels. As shown in Figure 7a, when the EMImAc content increased from 40 to 54 wt %, the ionic conductivity of RSF/EMImAc/H2O gels increased from 1.34 ± 0.37 to 8.69 ± 1.14 mS/cm, which is above the benchmark of 1 mS/cm desired for the use in electrochemical devices.45 On the other hand, the liquid electrolyte EMImAc was confined in a unique nanostructured network in the gel, which was constructed by the β-sheet of RSF and provided pathways continuous for ionic transport. The RSF/EMImAc/H2O gels with the low RSF content possessed high ionic conductivity because fewer frameworks facilitated ionic transport of the liquid electrolyte. As shown in Figure 7b, when the RSF content decreased from 10 to 5 wt %, the ionic conductivity of the as-prepared RSF/EMImAc/H2O gels increased from 1.34 ± 0.37 to 7.5 ± 1.16 mS/cm. Therefore, RSF/EMImAc/H2O gels with different conductivities can be prepared by varying the content of RSF and EMImAc. The promising conductivity combined with good air stability and tunable flexibility makes RSF/EMImAc/H2O gels good candidates as potential flexible conductive materials.

4. CONCLUSIONS

We developed a RSF-based gel (RSF/EMImAc/H2O gel or RSF/EMImAc/EtOH–H2O) with a unique nanostructured network, which displayed superior mechanical performance with tunable strength and flexibility, favorable conductivity, and remarkable air stability. The gel was formed by adding water (or ethanol–water) into the RSF/EMImAc solution to induce the conformation transition of RSF from random coil to β-sheet. The developed RSF-based gel demonstrated a significant improvement in its mechanical behavior, which is attributed to the unique homogeneous network structure constructed by large amounts of RSF nanofibers with a β-sheet formed during the conformation transition of RSF. The IL, EMImAc, not only played the role of a good solvent for silk fibroin to slow down the formation of the hydrophobic β-sheets dominated network in the RSF gel, but it also endowed
the gel with promising air stability because of its well-known capability for either adsorbing or desorbing the water according to the moisture content in the environment. Combining the conductivity of IL, such a "dried" and tough RSF/EMImAc/H2O gel showed great potential to be used as a multifunctional material, which could be dispensable with full encapsulation. However, it should also be mentioned that the RSF might forfeit the feature of biomaterials in this case because of the employment of IL.

**REFERENCES**


