Interfacial Rheology of Natural Silk Fibroin at Air/Water and Oil/Water Interfaces

Lijun Wang,† Hongen Xie,† Xiuying Qiao,*,† An Goffin,‡ Tom Hodgkinson,§ Xuefeng Yuan,§ Kang Sun,† and Gerald G. Fuller†

†State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, Shanghai 200240, China
‡Department of Chemical Engineering, Stanford University, Stanford, California 94305-5025, United States
§Manchester Interdisciplinary Biocentre, The University of Manchester, Manchester M1 7DN, United Kingdom

ABSTRACT: The interfacial viscoelastic behavior of natural silk fibroin at both the air/water and oil/water interfaces is reported. This natural multiblock copolymer is found to be strongly amphiphilic and forms stable films at these interfaces. The result is an interfacial layer that is rheologically complex with strong surface elastic moduli that are only slightly frequency-dependent. The kinetics of surface viscoelastic evolution are reported as functions of time for various concentrations of the spread films. Films deposited by Langmuir—Blodgett deposition were studied by scanning electron microscopy (SEM) and X-ray diffraction to reveal a fibrous structure at the interface. The production of stable O/W emulsions by silk fibroin further confirms the generation of the elastic films at the oil/water interfaces.

INTRODUCTION

Silk produced by the domesticated silkworm, Bombyx mori, has attracted recent attention because of its excellent mechanical properties, outstanding biocompatibility, low biodegradability, and minimal inflammatory reaction. These properties have led to the exploration of its use in biotechnological and biomedical applications, such as controlled drug release and tissue engineering scaffolds.1,2 Silk, as it is emitted from the glands of the silkworm, consists of a fibroin core (75 wt %) surrounded by gelatin-like sericin proteins (25 wt %), with the former constituent being a fibrous, structural protein with high crystallinity. As a result, fibers formed from this material display highly oriented crystalline alignment along the fiber axis.3–5 The sericin protein, with a large fraction of water-soluble serine and other hydrophilic amino acids, serves as a binder and aids silk fiber spinning by the animal. The sericin can be easily removed from cocoons to eliminate its adverse effects in medical applications.

The core protein of silk, silk fibroin (SF), is composed of highly repetitive amino acid sequences with alternating hydrophobic and hydrophilic blocks along the molecular chains and can be regarded as nature’s counterpart of a synthetic, multiblock polyelectrolyte. This multiblock structure allows the protein to self-assemble into micelles and form gels in concentrated solution.6,7 The relatively hydrophobic silk fibroin molecules consist of heavy and light chains of approximately 360 and 25 kDa in M_w, respectively, connected by disulfide linkages.8 The polypeptide chain is a known polymorph and can achieve at least three secondary conformations, two of which are stable and one of which is metastable. The two most commonly known conformations are called silk I and silk II, which exist as dimorphs of the fibroin from Bombyx mori. Silk I is a structure residing in solution in the glands of the silk worm before the spinning process.9,10 Silk II gives rise to the crystalline structure of fibroin in native silk fiber, with antiparallel β sheets crystallized in hydrophobic regions and more or less random conformations existing in hydrophilic regions.11 It is the unique coexistence of both crystallized hydrophobic pleated β sheets and amorphous hydrophilic regions in the fibroin structure that gives silk extraordinary mechanical properties with both high tensile strength and exceptional toughness. Silk III, reported by Valluzzi and Gido,12–14 is a crystalline structure formed at the interface of air and water. The formation of different secondary structures in fibroin depends on the charge density, pH, and salinity of its aqueous solvent and its processing conditions.

Silk fibroin possesses regions of different hydrophobicity when it folds into the appropriate secondary or higher-order structures. The coexistence of distinct hydrophobic and hydrophilic regions endows the silk fibroin molecules with amphiphilic character and surface activity, allowing silk fibroin to reside at fluid interfaces and form stable viscoelastic films at the surface of an aqueous medium and either air or oil. If this occurs, then the silk fibroin can be used as an emulsifier to form stable emulsions by creating viscoelastic shells on the surfaces of dispersed drops. Similarly, this mechanism can be used to stabilize foams effectively. These shells resist droplet or bubble deformation and prevent droplet and bubble coalescence and macroscopic phase separation. The purpose of this article is to report on the ability of

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silk fibroin to produce strong 2D gels at both air/water and oil/water interfaces.

Emulsion and foam stability depends on a complex array of phenomena, including droplet and bubble coalescence and Ostwald ripening. Coalescence involves film drainage and, ultimately, film rupture. Both processes will be strongly affected by the mechanical resistance of the films to shear and dilatational deformations as opposing films approach one another. Ostwald ripening, where species internal to drops and bubbles are transported across films, will also be controlled by thin film interfacial mechanical properties. Consequently, numerous investigations have focused on the development of methods to measure interfacial rheological properties faithfully\(^{15-19}\) and the consequences of these characteristics on emulsion and foam stability.\(^{20,21}\)

Like bulk fluids, fluid interfaces can also be characterized by the resistance to flow and a corresponding rheological response, which can provide insight into the absorption behavior and molecular interactions at different interfaces. Broadly, interfacial rheological properties are classified as either dilational or constant area properties. This article is concerned with interfacial, constant-area shear properties. The interfacial shear rheology, playing an important role in predicting long-term stability to coalescence, is very sensitive to the structure and composition of an adsorbed protein layer and the nature of intermolecular interactions in the interfacial film, both at air/water and oil/water interfaces.\(^{22}\) Different from simple amphiphilic molecules, proteins typically reconfigure upon adsorption to a fluid/fluid interface, and the resulting viscoelastic thin film can stabilize emulsions and foams.\(^{16}\)

There is very little reported use of silk fibroin as a stabilizer of emulsions and foams. Recently, it has been verified to be a successful stabilizer of corn oil—water emulsions, and the effects of pH, thermal processing, and salt concentration on the properties and stability of the resultant emulsions were investigated.\(^{23}\) However, the interfacial viscoelasticity and mechanisms for interfacial stabilization of silk fibroin have not been previously reported, and this article addresses these properties. In contrast to the work done by Valluzzi and Gido et al.,\(^{12-14}\) where films were formed through adsorption from solution (which also acted as the subphase), the study presented here demonstrates that stable films can be obtained by spreading a small amount of silk fibroin aqueous solution directly onto a phosphate-buffered saline (PBS) subphase. The interfacial shear rheological behavior of both air/water and oil/water interfaces in the presence of silk fibroin is discussed along with the morphology and microstructure of the adsorbed silk fibroin. In addition, direct evidence of emulsion stabilization using silk fibroin is offered along with the morphology and droplet size distribution of the resultant emulsions.

### EXPERIMENTAL SECTION

**Materials.** Fresh domestic *Bombyx mori* cocoon shells were kindly supplied by a farm cooperative in China. Analytical-grade sodium carbonate (Na\(_2\)CO\(_3\)), lithium bromide (LiBr), dodecane (≥99%), and hexanol (98%) were purchased from Sigma-Aldrich, butyl butyrate (98%) was purchased from Acros Organics, phosphate-buffered saline (PBS) 10X (pH 7.4) was purchased from Invitrogen, and all of the chemicals were used as received. The water used in this study was Milli-Q water.

**Preparation of Silk Fibroin Aqueous Solutions.** The preparation of silk fibroin aqueous solutions from fresh domestic *Bombyx mori* cocoon shells involved three steps: the extraction of sericin, the dissolution of the cocoon fiber, and the dialysis of the silk fibroin aqueous solutions against deionized water. The first step is the removal of sericin (degumming) from the cocoon shells by maintaining them in a 100 times by weight boiling 0.02 M Na\(_2\)CO\(_3\) aqueous solution for 30 min. The resulting silk fibroin material was then rinsed with deionized water. The degumming process was repeated at least three times, and the thoroughly degummed silk was then air-dried. The dissolution of silk fibroin was accomplished by stirring the resulting material in a 9.3 M LiBr aqueous solution at 80 °C until it was dissolved. This solution was then dialyzed against water (100 times by volume) for 2.5 h while refreshing the outer deionized water every 30 min. This procedure yielded very reproducible products of typically an 8 wt % silk fibroin aqueous solution, which was determined by weighing the remaining solid after the complete drying of the silk fibroin solution. By performing gel permeation chromatography with multiple light-scattering detectors, the molecular weight of silk fibroin was determined to be \(M_n = 2.96 \times 10^4\) g/mol with a polydispersity index of \(M_n/M_w = 1.12\). Before use, the fibroin solution was centrifuged at 13 000 rpm for 15 min to remove any aggregates, and then the solution was diluted with Milli-Q water to the desired fibroin concentration (\(\gamma_{SF}\)) for measurements. The diluted fibroin solutions were made fresh for each experiment.

**Preparation of Silk Fibroin Interfacial Layers by Langmuir—Blodgett Transfer.** Films of silk fibroin were deposited onto hydrophilic silicon substrates through Langmuir—Blodgett deposition. A Teflon trough was used, and 200 \(\mu\)L of a 20 mg/mL silk fibroin aqueous solution was spread onto the PBS solution surface and kept at rest for 2 h to ensure the achievement of an equilibrium state. After this time had transpired, the silicon substrates, which previously resided in the subphase, were extracted through the interface at a rate of 0.01 mm/s.

**Morphology Observation.** Specimens for morphology observation were obtained by Langmuir—Blodgett transfer. The films deposited on silicon substrates were examined using an FEI XL30 Sirion scanning electron microscope (SEM) operating at 2 kV to observe the morphology.

**Characterization of the Oil Phase.** The interfacial tensions of the oil phases were measured using a K100 tensiometer (Krüss tensiometer, Germany) with a Du Noisy ring arrangement.

**Interfacial Rheology Measurements.** Measurements of the interfacial rheology of silk fibroin at both air/water and different oil/water interfaces were obtained using the double wall-ring method described by Vanderbei et al.\(^{24}\) For this purpose, an ARG2 stress-control rheometer (TA Instruments, U.S.A.) was outfitted with a Du Noisy ring (radius \(R = 20\) mm) suspended from the upper geometry mount. The ring was placed concentrically within the gap of a PTFE cup that was machined to provide a double Couette effect of interfacial shear on either side of the ring. A cross section of the cup is provided in Figure 1.

Prior to the measurements, phosphate-buffered saline solution (PBS) was poured into the bottom of the cup to the level of the internal step shown in Figure 1. Aqueous silk fibroin solutions with different concentrations were spread dropwise onto the PBS solution surface using a microsyringe. The volume of solution spread at the surface of the subphase was adjusted so that the following surface concentrations were produced: \(\gamma_{SF} = 1, 2, 4, 8, 16\) mg/m\(^2\). For studies of different oil/water interfaces, dodecane, butyl butyrate, or hexanol with increasing polarity were placed on top of the fibroin layer by slow injection using a syringe to prevent the shearing of the spread silk fibroin film. Dynamic time sweeps were performed with a strain amplitude of \(\gamma_{SF} = 0.001\) (0.1%, in the linear regime) and an angular frequency of \(\omega = 1\) rad s\(^{-1}\) for 1 h until the fibroin formed a stable film at the interface. Dynamic frequency sweeps were operated with a strain amplitude of \(\gamma_{SF} = 0.001\) (0.1%, in the
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Morphology. The morphological features of silk fibroin at the air/water interface were observed by SEM following a single deposition as described in the Experimental Section. One such image is shown in Figure 2, where it can be seen that the protein produces oriented fibers of 10–80 μm in length and 0.1–0.75 μm in diameter. The orientation direction of the fibers is coincident with the draw direction of the silicon substrate through the interface and is a consequence of interfacial flow gradients that accompany the deposition process. In general, the average diameter of silk fibroin fiber immediately following the degumming process is around 20 μm as revealed using optical and scanning electron microscopy.26 Evidently, interfacial processing of the silk fibroin avoids bundling of the protein into thicker fibers. However, interfacial processing leads to higher polydispersity, and a network is formed as evidenced by the fiber–fiber interconnections. It is thought that this interfacial network formation is responsible for the 2D gel-like rheological response presented later. During the gelation process, the silk fibroin molecules cross-link onto one another physically and self-assemble into fibrous structures. However, the structure of silk fibroin at the interface may deviate from its native state, as suggested by Valluzzi et al.15

Interfacial Rheological Behavior at the Air/Water Interface. To trace the kinetics of structural reorganization of silk fibroin at the air/water and different oil/water interfaces, time sweeps were performed where the interfacial elastic and viscous moduli, \( G'_f \) and \( G''_f \), were measured at a fixed frequency of 1 rad \( s^{-1} \) and a strain amplitude of 0.1% (determined to be in the linear viscoelastic region) as functions of time. These measurements were repeated for different concentrations of silk fibroin in the surface layer, and the results are shown in Figure 3 for the air/water interface.

In comparison with low-molecular-weight surfactants, proteins diffuse slowly and absorb more slowly to the interface. Once they are absorbed to the interface, they start to unfold, change conformation, and interact strongly with neighboring molecules to form a 2D viscoelastic gel.26 It can be seen from Figure 3 that the elastic modulus, \( G'_f \), abruptly rises and then increases to steady values over timescales on the order of tens of minutes. In addition, the time required for \( G'_f \) to achieve steady values increases with concentration. The viscous modulus, \( G''_f \), is an order of magnitude lower and, following an initial increase, does not change with time. The values of surface elastic moduli exhibited by silk fibroin are very large in comparison with those of proteins such as lysozyme, insulin, and \( \beta \)-casein21,27 and have the characteristics of a strong interfacial gel. The kinetics of surface elasticity development, showing a two-step process with a very rapid initial rise in the elastic modulus followed by a slower, further increase to steady values offer insight into the process of molecular conformational change and the intermolecular self-assembly of silk fibroin at the air/water interface. Evidently, the prevalence of silk \( \beta \)-sheets undergoes a rapid, initial development that is followed by a slower, gelation transition as physical cross-links form. As the silk fibroin concentration increases, the elastic moduli measured after 60 min, \( G'_f |_{600} \) increase until a concentration of approximately 4 mg/m\(^2\) is reached, as shown in Table 1. Past this concentration, the elastic moduli are insensitive to concentration. This concentration dependence is substantially different from the response of silk fibroin hydrogels, which demonstrate a much stronger dependence on the protein concentration.

RESULTS AND DISCUSSION

Preparation and Characterization of Silk Fibroin Emulsions. By the use of an FM200 high-shear dispersing emulsifier (Fluko Equipment Shanghai, China), emulsions were prepared by mixing aqueous silk fibroin solutions with volumes of oil at a speed of 10,000 rpm for 2 min. Silk fibroin concentrations of greater than 20 mg/mL led to excessive foaming, and fluidity was lost when the oil volume fraction, \( \phi_v \) exceeded 0.6. For these reasons, three fibroin concentrations (\( \phi_s = 2.5, 5, \) and 10 mg/mL), three oil volume fractions (\( \phi_v = 0.2, 0.3, \) and 0.4), and three oil phases (dodecane, butyl butyrate, and hexanol) were chosen for emulsion preparation in this study.

After preparation, the emulsions were poured into standard serum bottles for stability analysis by observing the change in the emulsion—aqueous-phase interface and recording the volume fraction changes in the stable emulsions with time. The size and size distribution of the emulsion droplets were estimated by utilizing a Mastersizer 2000 (Malvern, U.K.) at a refractive index (RI) of 1.450, and the laser obscuration was about 10%.

Figure 1. Interfacial shear rheology setup: the oil phase is slowly injected onto the PBS surface in the presence of silk fibroin, and the Du Noüy ring resides at the oil/PBS interface and air/PBS interface. (1) PTFE cup, (2) PBS solution, (3) oil phase, and (4) silk fibroin interfacial layer. The dashed curve represents the internal step.

Figure 2. SEM image (magnified by 5000) of the silk fibroin interfacial layer prepared from the air/water interface by Langmuir–Blodgett transfer, and the arrow indicates the direction of pulling for the silicon substrate with a deposited layer of silk fibroin from the air/water interface.
concentration ($\varphi$) of $G' \propto \varphi^{1.5}$ at low protein concentration $0.32 < \varphi < 1.3$ wt %) and $G' \propto \varphi^{0.5}$ in the entangled regime at high protein concentration $1 < \varphi < 5.2$ wt %). The fact that the surface elastic moduli appears to saturate at concentrations above 4 mg/m$^2$ suggests that the interfacial layers support only a finite amount of fibroin protein and that further increases in fibroin concentration simply send excess protein into the bulk.

Figure 4 is a compilation of the frequency sweeps of interfacial elastic and viscous moduli of the samples measured after a 60 min incubation time of the layers deposited at various silk fibroin concentrations. Over the measured frequency range, both the elastic and viscous surface moduli are essentially independent of frequency, a trend that has also been observed for other surface-active biopolymers and proteins.\(^{29}\) Silk fibroin achieves remarkable elastic-like behavior with $G'_e > G''_e$ at all frequencies, regardless of concentration. These results imply that the $\beta$-sheet crystallites organized by the silk fibroin molecular chains display gel-like behavior,\(^{28}\) consistent with soft glass rheology.\(^{18}\) In Figure 4c, the complex surface viscosity is plotted against frequency and reveals the apparent pseudoplastic behavior of silk fibroin with continuous power-law behavior of $[\eta_0^*] \propto \omega^{-(0.93 - 1.0)}$ for all concentrations.

Strain-sweep experiments were conducted to investigate the possible fracture mechanisms of silk fibroin films. The strain dependence of interfacial elastic modulus $G'_e$ is shown in Figure 5a. The strain sweeps indicate a regime of linear viscoelasticity below a strain of 1%, following which the elastic modulus undergoes a precipitous drop with the occurrence of some breakage in the silk fibroin interfacial gel structure. It is evident that a higher concentration of silk fibroin helps to enhance the interfacial elasticity, which leads to a greater interfacial storage modulus, a broader linear viscoelasticity region, and a slower modulus decrease in the nonlinear region. The recovery of the silk fibroin layer structure following the fracture of the gels in the strain sweeps described above was investigated by repeating a second dynamic time sweep measurement immediately following first strain fracture. These are shown in Figure 5b, and the plots indicate that the fibroin layers are able to recover partially, but only to a level of 7–50% of the original steady values of $G'_e$ as shown in Table 1.

**Interfacial Rheological Behavior at Oil/Water Interfaces.**

As described previously, the stabilization of emulsions is sensitive to the composition of the interfacial film formed at the oil/water interface. The polarity of the oil phase not only affects the emulsion stability\(^{30}\) but also influences the properties of the interfacial layer formed between the oil phase and the aqueous phase. Different oil phases are generally characterized by their interfacial layer formed between the oil phase and the aqueous phase. Different oil phases are generally characterized by their chain length and polarity.\(^{31}\) In this work, three oil phases of dodecane, butyl butyrate, and hexanol leading to increased oil chain length and polarity were used. Following El-Mahrab-Robert et al.,\(^{32}\) we use the interfacial tension, $\gamma_i$, to rank the polarity of the oils. As indicated in Table 2, the interfacial tension

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**Table 1. $G'_e$ of the Air/Water Interface and Different Oil/Water Interfaces in the Presence of Silk Fibroin with Different Concentrations ($\varphi_{SF}$) after 60 min in the First Time Sweep and the Second Time Sweep**

<table>
<thead>
<tr>
<th>Interface</th>
<th>$\varphi_{SF}$ (mg/m$^2$)</th>
<th>$G'<em>e</em>{60}$ first time sweep (mN/m)</th>
<th>$G'<em>e</em>{60}$ second time sweep (mN/m)</th>
<th>Recovery percentage for the second time sweep (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>air/water</td>
<td>1</td>
<td>9.4</td>
<td>0.7</td>
<td>7.4</td>
</tr>
<tr>
<td>air/water</td>
<td>2</td>
<td>80.6</td>
<td>14.7</td>
<td>18.2</td>
</tr>
<tr>
<td>air/water</td>
<td>4</td>
<td>144.1</td>
<td>19.1</td>
<td>13.3</td>
</tr>
<tr>
<td>air/water</td>
<td>8</td>
<td>160.0</td>
<td>84.7</td>
<td>52.9</td>
</tr>
<tr>
<td>air/water</td>
<td>16</td>
<td>158.1</td>
<td>46.5</td>
<td>29.4</td>
</tr>
<tr>
<td>butyl butyrate/water</td>
<td>1</td>
<td>18.6</td>
<td>11.7</td>
<td>62.9</td>
</tr>
<tr>
<td>butyl butyrate/water</td>
<td>2</td>
<td>68.3</td>
<td>47.9</td>
<td>70.1</td>
</tr>
<tr>
<td>butyl butyrate/water</td>
<td>4</td>
<td>86.2</td>
<td>62.3</td>
<td>72.3</td>
</tr>
<tr>
<td>butyl butyrate/water</td>
<td>8</td>
<td>121.3</td>
<td>67.3</td>
<td>55.5</td>
</tr>
<tr>
<td>dodecane/water</td>
<td>4</td>
<td>132.2</td>
<td>98.8</td>
<td>74.7</td>
</tr>
<tr>
<td>hexanol/water</td>
<td>4</td>
<td>21.0</td>
<td>7.3</td>
<td>34.8</td>
</tr>
</tbody>
</table>
values of dodecane, butyl butyrate, and hexanol are 55.3, 18.8, and 5.0 mN/m, respectively, which means that these oils are ranked as dodecane < butyl butyrate < hexanol, with the highest polarity and solubility belonging to hexanol (C6H14O).

The time evolution of \(G'_s\) and \(G''_s\) of silk fibroin at the oil/water interface is shown in Figures 6b and 7b for several concentrations of the protein. In most respects, the qualitative and even quantitative responses of the moduli are similar for both the air/water and oil/water interfaces. One difference is that the fibroin layers at the oil/water interfaces continue to increase slowly and take a much longer time to attain equilibrium than the air/water interface, especially for the layers with a higher fibroin concentration and for the more polar oil phases. This suggests that the increased hydrophobicity of the oil/water interface encourages the microstructural rearrangement of the fibroin that induces a slow enhancement of physical cross-links, but with the increase in the oil polarity and the resultant decrease in the molecular interactions between hydrophobic domains of fibroin and oil, the surface moduli of fibroin layers (\(G'_s, \sigma_0\)) are strongly reduced from 132.2 to 21.0 mN/m at the hexanol/water interface.

Table 2. Surface Tension and Interfacial Tension of Pure Water and Oil Phases

<table>
<thead>
<tr>
<th>liquid</th>
<th>surface tension (mN/m)</th>
<th>interfacial tension (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>72.6</td>
<td></td>
</tr>
<tr>
<td>dodecane</td>
<td>25.2</td>
<td>55.3</td>
</tr>
<tr>
<td>butyl butyrate</td>
<td>12.0</td>
<td>18.8</td>
</tr>
<tr>
<td>hexanol</td>
<td>26.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Figure 4. Frequency sweep experimental results of silk fibroin stabilized at the air/water interface for 60 min: (a) double-logarithmic plots of the interfacial storage modulus vs frequency; (b) double-logarithmic plots of the interfacial loss modulus vs frequency; and (c) double-logarithmic plots of the interfacial viscosity vs frequency.

Figure 5. (a) Strain dependence of interfacial storage modulus \(G'_s\) of silk fibroin stabilized at the air/water interface for 60 min. (b) Time evolution of the interfacial storage modulus \(G'_s\) of silk fibroin at the air/water interface obtained from the second time sweep immediately after the nonlinear strain fracture.
Further evidence that oil/water interfaces promote the conformational restructuring of fibroin changes to take advantage of enhanced hydrophobicity comes from the frequency dependence of the surface moduli. Similar to the air/water interface, which produced a relatively flat frequency response, presenting fibroin to an oil/water interface leads to frequency-independent $G'_s$ and $G''_s$. The dynamic surface viscosity, however, is still inversely proportional to frequency, as in the case of the air/water interface (data not shown).

Finally, the strain sweeps of the films at the oil/water interface in Figures 6a and 7a also show only a modest change from the air/water interface with a slight increase in the maximum strain to induce a modulus reduction. It seems that the silk fibroin film generated at the air/water interface is more fragile than that at the oil/water interface, especially the dodecane/water interface, with a shorter linear viscoelastic region and a faster decrease in $G'_s$ at the same strain in the nonlinear viscoelastic region. This phenomenon is attributed to the strengthening of the interfacial gel at the oil/water interface because of the affinity of hydrophobic domains of silk fibroin and oil molecules, which also leads to a much higher recovery (nearly 70%, except for the hexanol/water interface) of the interfacial elasticity than at the air/water interface after the removal of the external nonlinear strain, just as reflected by Table 1 and Figures 6b and 7b. The interfacial toughness and interfacial elasticity recovery both increase as the polarity decreases and the resultant enhancement of the molecular interactions of fibroin and oil at the interface occurs. At the oil/water interfaces and the dodecane/water interface in particular, nonlinearities at large strains may arise from the sliding of β sheets instead of fragment breakage. Obviously different from the air/water interface, silk fibroin interfacial films can sustain larger deformations without structural fracture (up to strain amplitudes of 1%) than proteins such as soy protein isolates33 but are noticeably weaker than biopolymers such as acacia gum.29

Large differences in oil polarity might be expected to lead to different levels of solubilization and changes in interfacial protein conformation.34–37 The higher interfacial modulus of the silk fibroin film formed at the dodecane/water interface compared to that of the butyl butyrate/water and hexanol/water interfaces suggests a higher entanglement density for this solvent. Dilational rheology measurements have demonstrated that protein films at oil/water interfaces are more expansible and compressible compared to the air/water interface,2 with the major difference being that oils are better solvents for the more hydrophobic residues of the protein polypeptide chains. In a

Figure 6. (a) Strain dependence of interfacial storage modulus $G'_s$ of silk fibroin stabilized at the butyl butyrate/water interface for 60 min. (b) Time evolution of the interfacial storage modulus $G'_s$ of silk fibroin at the butyl butyrate/water interface obtained from the first time sweep and the second time sweep immediately after the nonlinear strain fracture. The inset plot with time on the logarithmic scale is added for easier discrimination.

Figure 7. (a) Strain dependence of interfacial storage modulus $G'_s$ of silk fibroin stabilized at different oil/water interfaces for 60 min. (b) Time evolution of interfacial storage modulus $G'_s$ of silk fibroin at different oil/water interfaces obtained from the first time sweep and the second time sweep immediately after the nonlinear strain fracture.
recent study by Maldonado-Valderrama et al., the effect of gastric conditions (pH, temperature, and ionic strength) on β-lactoglobulin films at different fluid interfaces of air/water, tetradecane/water, and olive oil/water was investigated, where tetradecane had a higher interfacial tension (53.0 mN/m, lower polarity) than olive oil (29.5 mN/m, higher polarity). The experimental results revealed that the less-polar tetradecane enhanced the adsorption and conformational changes in the protein because of its stronger preference for hydrophobic domains of protein, causing a greater dilatational modulus compared to that of olive oil.

Protein adsorption is naturally dependent on the attributes of the protein, such as the molecular weight, hydrophobicity, and secondary structure. Although our findings of fibroin interfacial moduli share much in common with other proteins, such as lysozyme (14.5 kDa) and insulin (6 kDa), and it exhibits larger interfacial moduli. In addition, lysozyme is a compact globular protein, and silk fibroin may be a more extended protein with a greater affinity to adsorb. Consequent lysozyme shows faster molecular arrangement at an interface and undergoes a faster interfacial gelation process.

Stability, Morphology, and Droplet Size of the Fibroin-Stabilized Emulsions. To confirm the emulsification effectiveness of silk fibroin, oil-in-water emulsions were prepared in the presence of fibroin at various concentrations. The residual emulsion volume fraction with time and the appearance of the emulsions 7 days following emulsion preparation are shown in Figure 8. The volume fraction of the residual emulsion was determined by dividing the volume of the emulsion phase by the total initial emulsion volume. It can be seen in Figure 8 that the emulsions reach steady state after experiencing an initial increase in the resolved water fraction and a decrease in the residue emulsion fraction with the time. It is evident that silk fibroin is an effective stabilizer, presumably because of the strong interfacial elastic moduli that suppress droplet coalescence.

The fibroin concentration and oil-phase polarity influence the emulsion stability, and the oil volume fraction has no obvious effect on it. Although increasing ϕ_SF was not observed to reduce the onset interfacial gelation time, increasing this parameter decreases the time needed to achieve equilibrium (10% of the total initial emulsion volume). It is evident that silk fibroin is an effective stabilizer, presumably because of the strong interfacial elastic moduli that suppress droplet coalescence. The fibroin concentration and oil-phase polarity influence the emulsion stability, and the oil volume fraction has no obvious effect on it. Although increasing ϕ_SF was not observed to reduce the onset interfacial gelation time, increasing this parameter decreases the time needed to achieve equilibrium (10% of the total initial emulsion volume).

Optical microscopy and light-scattering analysis were used to analyze the morphology and size of the emulsion droplets. Just as reflected in Figure 8a, not all of the emulsion droplets are spherical, and indeed some droplets have elliptical or even more intricate shapes. Generally, a fluidlike interfacial layer will allow the interface to relax toward a spherical shape to minimize the surface energy. However, due to the large surface area of the fibroin-laden droplets, the fibroin interfacial layer will be decreased and the corresponding interfacial toughness. However, it seems that the oil polarity plays a more important role in the final stable emulsion fraction than does the oil structure, and the increase in oil polarity obviously increases the amount of the emulsion after reaching the steady state. It should be noted, however, that the data in Figure 8 provide an indication of the short-term stability but not the long-term stability of the emulsions. However, all of these emulsions were observed to be very stable over long time periods (several months).

The average droplet sizes reported in Table 3 decrease with an increase in ϕ_SF but increase with an increase in ϕ_o. Among the O/W emulsions stabilized by silk fibroin with different oil phases, the average droplet size of the tetradecane/water emulsion appears to be the greatest. The increase in the fibroin concentration enhances the interfacial elasticity and mechanical strength and suppresses the flow-induced coalescence, leading to smaller drop sizes. However, for a fixed fibroin concentration, with the increase in the oil volume fraction and the resultant increase in the oil droplet surface area, the adsorbed amount of silk fibroin at the oil/water interface will be decreased and the corresponding...
decrease in the strength of the interfacial gel structure will influence the droplet stability. After Ostwald ripening, the droplet size increases because of the weakening of the protective ability of interfacial layers with respect to droplet deformation. The decrease in the droplet size with the increase in oil polarity should be attributed to the decrease in the interfacial tension of the oil/water interface.

**CONCLUSIONS**

The coexistence of distinct hydrophobic and hydrophilic regions endows natural silk fibroin molecules with amphiphilic character and surface activity, making this molecule able to form stable viscoelastic films at the interface of an aqueous medium with either air or oil. This character suggests that silk fibroin could have important potential applications in drug-delivery systems and cosmetics as an effective emulsion stabilizer because of its excellent mechanical properties and biocompatibility. At both air/water and oil/water interfaces, silk fibroin can form stable, solidlike interfacial gels with ordered β-sheet structures after protein conformation rearrangement and intermolecular self-assembly at the interface. The resulting interfacial moduli are remarkably large and increase with the silk fibroin concentration. Because of the increased compatibility between the hydrophobic domains of silk fibroin and oil molecules, the silk fibroin interfacial gel generated at the oil/water interface is more stable, stronger, and tougher than that at the air/water interface, possessing a greater interfacial yield strain and a higher recovery ability after the nonlinear fracture. It is verified that silk fibroin can stabilize the O/W emulsions with oils of different polarities, and increasing fibroin concentrations and oil polarities are helpful for attaining more stable emulsions. The average droplet size decreases with the increase in oil concentration or oil polarity and the decrease in oil volume fraction.

**AUTHOR INFORMATION**

**Corresponding Author**
*E-mail: xyqiao@sjtu.edu.cn.*

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