Liquid Exfoliated Natural Silk Nanofibrils: Applications in Optical and Electrical Devices

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Natural polymeric materials (e.g., silk, cellulose, chitin, etc.) consist of sophisticated hierarchical architectures spanning the nanoscale to macroscale, resulting in utility as engineering and decorative materials for thousands of years.[1] The applications of these materials have been extended to different fields, such as biomedicine,[2] photonics,[3] and electronics.[4] A variety of methods[5] have been developed to extract natural biopolymer nanomaterials to retain the nanostructures with the aim of enhancing the properties.[1b,d,6]

These approaches have included physical (e.g., high pressure homogenizers,[5e–h] grinders/refiners,[5i] cryocrushing,[5j,k] and high intensity ultrasonic treatment[5l,m]) chemical (e.g., acid hydrolysis,[5n–q] 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO) oxidation,[5r–u] and ionic liquid dissolution[5v,w]) and biological (e.g., enzymatic[5x,y]) methods to extract cellulose and chitin to obtain nanomaterials, including nanocrystals, nanowhiskers, and nanofibrils.[1b,d,5a–d] However, direct extraction of silk nanofibrils (SNFs) from natural silk fibers remains a challenge due to the high crystallinity and complex hierarchical structure. To date, a chemical method (formic acid/CaCl₂ dissolution[5j]) and a physical method (ultrasonication[5j]) have been reported but both with intrinsic limitations.

Formic acid/CaCl₂ dissolution generates SNF bundles or aggregates and these SNFs are only stable for a short time (less than 6 h) due to the dissolution imposed by the formic acid. For the ultrasonic method, silk fibers are only exfoliated to generate SNF mats and lack re-processing, thus limiting the regeneration of materials.[5] More importantly, both methods do not exfoliate silk fibers on the single SNF scale, which is crucial when defining the mechanical properties of silks and is helpful as a starting point for the regeneration of new materials.

We were inspired by liquid exfoliation of graphite,[9] a method that directly exfoliates bulk layered graphite into 2D graphene sheets in organic solvent, without the need for chemical oxidation of the graphite (Figure 1). Here, we elaborate a new strategy to directly exfoliate silk fibers on the single SNF level, by integration of chemical (partial dissolution) and physical (ultrasonic dispersion) methods (Figure 1), creating a new method for silk fiber processing. Finally, we show how the exfoliated SNFs, which retain their natural structure and properties, can be used to produce free standing nanomaterials with biocompatibility and useful optical properties.

Figure 2a summarizes the three-step top-down route to exfoliate natural silk fibers. First, the degummed Bombyx mori (B. mori) silkworm cocoon silk fibers were immersed in hexafluorosopropanol (HFIP) solution with a weight ratio of 1:30 and were incubated in an airtight container as a silk fiber/HFIP mixture at 60 ºC for 24 h. During the incubation, the HFIP gradually permeated into the silk fibers and partially dissolved the silk from defects and ends (Figure 2b and Figure S1, Supporting Information). After 24 h, the silk fiber/HFIP mixture formed a pulp blend (Figure 2a) and was split to microfibrils with diameters of 5–50 µm and contour length of 50–500 µm (Figure 2b). Scanning electron microscopy (SEM) images revealed that these silk microfibrils (SMFs) were also split off to sub-microfibrils at their ends (Figure 2b). The dried SMFs were moved into water with a weight ratio of 1:200. The undissolved silk fibers and large SMFs (contour length larger than 1 mm) twisted together and settled to the bottom after 1 min stirring or shaking (Figure 2a). Most of the SMFs remained in water during 1 h. In the third step, the settled silk fibers were removed, and ultrasonication was applied to exfoliate the SMFs into SNFs. After 1 h of ultrasonic dispersion at 120 µm amplitude, 20 kHz frequency and 20 min centrifugation at 10 000 rad to remove the precipitates, the transparent well-dispersed SNFs were obtained and were stable over several months (Figure 2a). The extracted SNFs had a diameter of 20 ± 5 nm and a contour length in the range of 300–500 nm (Figure 2b and Figure S2, Supporting Information), same as the diameters of single SNFs found in native silk fibers.[10] Typically, 1 g of degummed silk fibers produced 100 mL SNFs aqueous solution with concentration of ≈0.1 wt% after 1 h of ultrasonic dispersion at 120 µm amplitude and 20 kHz frequency, giving a yield around 10%. The settled silk fibers and SMFs could be dissolved and dispersed again to generate higher yields of SNFs. The more detailed discussion of optimum conditions for this liquid exfoliation method can be found in Figure S3 (Supporting Information). Notably, this liquid exfoliation method is not only suitable for B. mori silk fibers, but also works for the formation of other biological nanofibrils from bulk materials. For example, by using the same method, we have successfully extracted Antheraea pernyi (A. pernyi) SNFs and chitin nanofibrils from A. pernyi silkworm silk fibers and crab shell α-chitin powder, respectively (Figure S4, Supporting Information).

To help understand the mechanism involved in the liquid exfoliation of silk fibers, a coarse-grained dissipative particle dynamics (DPD) simulation was applied to the process (Figure 3). According to the classic micelle model of silk...
Figure 1. Schematic representation of a new strategy followed to liquid exfoliation of silk fiber. Such a method is inspired by liquid exfoliation of graphite, which directly exfoliates bulk layered graphite into 2D graphene sheets in the organic solvent without the need for chemical oxidation of the graphite.

Figure 2. Liquid exfoliation process of silk fiber. a) Schematic and photographs showing the fabrication steps and their relevant solution. Step 1: silk fiber was immersed in HFIP solution with weight ratio of 1:30 and the mixture was incubated for 24 h to obtain silk fiber/SMFs slurries. Step 2: the dried silk fiber slurries were transferred to H$_2$O solution and the silk fiber precipitation was removed to gain SMFs dispersion. Step 3: the SNFs dispersion was prepared via ultrasonic treatment. b) Schematic (top row) of liquid exfoliation of silk fiber and representative optical microscopy (the first two images in middle row) and scanning electron microscopy (SEM; the third image in middle row, and the images in bottom row) images of resultant products in each process. Silk fiber was easily dissolved by HFIP from the defect and end part, and can be split to SMFs after 24 h. The white arrows in optical microscopy and SEM images show the dissolution parts of silk fibers and SMFs. The SNFs with a diameter of 20 ± 5 nm can be obtained after ultrasonic dispersion (the SEM images in the last column).
assembly (Figure 3a). The crystalline regions (repetitive GAGAGS motifs) of silk form cores of fibroin micelles due to strong hydrophobic interactions and amorphous peptide chains (nonrepetitive motifs) which extend out from the core domains to form outer adhesion regions between fibroins. These micelles can assemble into SNFs and then silk fibers during spinning by elongational flow and/or physical shear (Figure 3a). The outer amorphous (hydrophilic) regions have much weaker interactions in comparison to the strongly bound crystalline cores (hydrophobic). Thus the exfoliation of the silk fibers was proposed occurring at these outer adhesion regions composed of the more hydrophilic peptide chains.

To verify this hypothesis, a molecular model was built to study the dynamics of silk chains under ultrasonic liquid exfoliation. Details of the model parameters are included in the Experimental Section of the Supporting Information. All the simulations were performed using the large-scale atomic/molecular massively parallel simulator. Decapeptide chains, each composed of 30 amorphous beads, were constructed to represent the inter-globule amorphous regions in the silk fibers, which were immersed in a simulation box filled with water beads. After equilibration of the system, the simulation box was deformed sinusoidally to generate a pressure perturbation in order to mimic the ultrasonication condition (for condition details, see the Experimental Section of the Supporting Information). The pressure perturbation and frequency in the simulations were much higher than those in the experimental conditions, but were utilized to provide a qualitative understanding of the molecular scale ultrasonication dynamics. The recording of the radius of gyration ($R_g$) and snapshots of the simulation (Figure 3b) showed the evolution of the peptide chains from a condensed assembly to separate chains. During this process, the periodic deformation pumped the water beads into the structure to collide with silk beads and squeeze into spaces between the amorphous chains to result in their separation. After the disruption, the peptide chains were dispersed in the liquid. The same procedure was performed on the hydrophobic chains (Figure 3c). Because of the strong attractive interactions (hydrogen bonds formed in the crystalline $\beta$-sheet) between the hydrophobic beads and the unfavorable interactions with the water beads, the hydrophobic chains did not disperse under the same condition in which the hydrophilic regions of the assemblies were separated. Therefore, ultrasonic exfoliation disrupted the amorphous regions that linked SNFs together. Our recent simulation work also indicated that the spinning process in producing silk fibers enhanced the connections along the fiber direction, while weaker entanglements were formed in the lateral directions. Considering the weaker connections between fibroin micelles in the lateral direction, we propose that the ultrasonication exfoliate the silk fibers bundles along the fiber direction (Figures 1 and 2).

In order to fabricate macroscopic materials, a vacuum filtration process was utilized as reported previously. Since SNFs have long contour lengths and strong mechanical properties, they can withstand vacuum-filtration drying and form homogeneous membranes (Figure 4a). After complete drying, the membranes (thickness ≈ 5 µm) appeared homogeneous, free-standing, and transparent (Figure 4b), and were characterized by strong birefringence in cross-polarized light (Figure 4c), indicating the ordered nematic phase in aqueous suspensions of SNFs (Figure S2d, Supporting Information) was maintained during the film formation process. Similar results were observed for other $\beta$-sheet-based nanofibril membranes.

![Figure 3. The potential exfoliation mechanism disclosed through DPD simulation. a) Model of hierarchical self-assembly and structures of B. mori silk fibers. b) The radius of gyration ($R_g$) of the hydrophilic chains as a function of simulation time. The increase indicates the initial collection of chains falls apart under ultrasonication (insets). c) The $R_g$ of the hydrophobic chains remains constant with simulation time. Snapshots show that the collection of chains does not fall apart under ultrasonication (insets).](image-url)
The Fourier transform infrared spectroscopy (FTIR) spectrum of SNF membrane (Figure S5, Supporting Information) confirmed that HFIP was totally removed from the SNF membranes.

Structural insights into the mesoscopic structure of the membranes from surface and cross-sections were obtained via SEM. A uniform fibrous and connected porous structure was evident with pore sizes of 5–20 nm (Figure 4d–f). Traditionally, as cast silk fibroin (SF) membranes dissolve in water if not treated with alcohol or by water annealing (Figure S6a, Supporting Information). Yet, these SNF membranes could be immersed in water and did not undergo dissolution for more than 1 week (Figure S6b, Supporting Information). To examine the structural details, the FTIR spectrum of SNF membrane was deconvoluted according to previous reports (Figure S7, Supporting Information). The deconvolution of the amide
I band provided an estimation of β-sheet (crystalline) structure in the SNF membranes at 53 ± 2%, while that of the degummed silk fibers was 38 ± 4%. Therefore, the SNF membranes had a higher content of β-sheet, acting as crosslink points to form interlocking protein chains to keep the SF molecules stable in solvent. These results also suggest that the HFIP exfoliation process mainly impacted the random coil structures in the silk fibers, which is consistent with our suggested liquid exfoliation mechanism.

Tensile tests were carried out to measure the mechanical properties of the materials (Figure S8, Supporting Information). Membranes with a thickness of 200 ± 25 µm had a modulus of 3.5 ± 0.3 GPa, higher than that of regenerated SNFs (2.5 GPa\(^{[14]}\)), silk fibroin membranes (1.5–2.7 GPa\(^{[14a,18]}\)), and chitin fibrils (1.3–2.3 GPa\(^{[19]}\)), while comparable with other types of β-sheet-based fibrils (2–5 GPa\(^{[20]}\)). As for toughness, the SNF membranes had values of 5 ± 2 × 10\(^5\) J m\(^{-3}\), 5–100 times higher than that of regenerated SNFs and other types of β-sheet-based fibrils (4 × 10\(^3\) to 9 × 10\(^4\) J m\(^{-3}\)\(^{[14b]}\)).

The cytocompatibility of SNF membranes was evaluated in vitro by seeding human dermal fibroblasts (HDFs). Ethanol-treated membranes were cast from aqueous SF solutions to serve as a positive control, as the cytocompatibility of aqueous-derived SF materials has been demonstrated in the past.\(^{[3a,21]}\) Alamar blue assay was performed to determine the proliferation of cells cultured on both types of membranes. Cells showed a linear progression of proliferation up to day 7 followed by a plateau. In comparison to the SF membranes, no significant differences in cell proliferation were observed (Figure 4g).

To evaluate the transmittance of SNF membranes, an ≈200 µm thick membrane was characterized via a UV–vis spectrophotometer (Figure 5a,b). The membrane was optically transparent (above 70% transmission) throughout the visible region (300–800 nm), and up to 88% at 800 nm (Figure 5a,b).

**Figure 5.** SNF-based optical membranes. a) A photograph to illustrate the transparency of SNF membrane. The inset is a photograph of a silk fibroin membrane after 70% ethanol treatment. b) UV–vis transmittance of silk fibroin (SF) and SNF membranes ≈200 µm thick. c) The fluorescent “MIT” letters fabricated by SNF membranes under visual (top) and UV light (bottom). Letters “M” and “T” were prepared by SNF/Rhodamine B and SNF/Rhodamine 123 dispersion, respectively. Letter “I” was prepared by SNF dispersion without any fluorescent dyes. d) Quantum dots patterned SNF membrane under visual (left) and UV light (right). All scale bars are 1 cm; (c) and (d) have the same scale size.
These transmission values were higher than the as cast (73% at 800 nm) and ethanol-treated (60% at 800 nm) SF membrane (inset of Figure 5a,b) with similar thickness, and comparable with transparent polymeric membranes prepared from polycarbonate (89%) and poly(methyl methacrylate) (92%).

In addition, silk fibroin, as an amphiphilic polymer, constituted by chains containing alternating hydrophobic and hydrophilic domains (Figure 3a) can absorb different types of dye to generate membranes with different colors. Colored luminescent SFN membranes with “M” and “T” letter patterns were colored by rhodamine B and rhodamine 123, respectively (Figure 5c). To verify the luminescent properties of silk fibroin with ultralow dye concentrations, the colored letter “M” was prepared with Rhodamine B ($1 \times 10^{-9} \text{ M}$) at a concentration 1000 times lower than that of Rhodamine 123 ($1 \times 10^{-6} \text{ M}$). The letter “M” was transparent under visible light, but showed bright red luminescence under UV light.

Additionally, the SFN membranes were able to take up different kinds of quantum dots to develop transparent optical nanodevices. CdSeS/ZnS quantum dots were patterned on the SFN membranes through masked vacuum filtration (Figure 5d). The membranes were also transparent with no icon observed under visible light, while the bright green icon appeared once the membrane was illuminated under UV light. These approaches to functionalize silks provide options toward bio-optic, packaging, and anti-counterfeiting devices.

Besides the application in optical devices, the SFNs can also be constructed to flexible electronic devices. The steps of fabricating SFN-based electric devices are illustrated in Figure 6a. The approach is different from routine methods, such as transfer printing and atomic deposition, which require complicated design and preparation processes. Instead, vacuum-assisted filtration was used to fabricate patterned SFN electronic devices. First, large single gold crystal platelets, for conduction, were synthesized on the exfoliated SFNs at pH 1. Chloroauric acid was used as the source of gold and the SFNs were the reductant (the details of synthesis can be found in the Experimental Section of the Supporting Information). The synthesized gold nanoplatelets are also suitable for biological applications. Figure 6b presents gold colloidal suspensions formed based on 0.05 wt% SFNs and 0.1 wt% gold nano platelets (based on 100% reduction yield). The suspension possesses a...
characteristic golden color with a shining surface due to the reflection of light. The SEM image (Figure 6c) revealed that the synthetic gold nanoplatelets were hexagonal, triangular, and polyhedral with lateral sizes up to 10 μm. Next, the gold nanoplatelet suspensions were added to a patterned mold, which was supported by a vacuum filtration membrane. After the gold nanoplatelet suspension was dried, the mold was removed, and the SNF dispersion was added to the filtration bottle. The patterned conductive SNF electronic devices could be obtained after drying (Figure 6d,e).

The moss green solid line in Figure 6f presents the nonlinear relationship between weight and volume composition of gold platelets in the membranes that arose from the large density mismatch between gold and protein (gold has a density about 14 times higher than the protein.24). At low gold content, the volume fraction weakly depends on the weight fraction of gold. By increasing the gold content, this dependence becomes stronger. Accordingly, the electric conductivity of these SNF/gold nanoplatelet membranes was tunable by changing the weight (or volume) ratio of gold nanoplatelets and SNFs used in the process. The membranes generated insulating-like in-plane conductivities for gold contents ≤74 wt% (or 15.3 vol%) (conductivity <10⁻⁸ S cm⁻¹, the white region in Figure 6f), while metallic conductivities were about 10¹ S cm⁻¹ with the threshold composition of 74 wt% (or 15.3 vol%) gold nanoplatelets (the shadow region in Figure 6f). These results were similar to amyloid/gold hybrids24 (the threshold composition of 87 wt%), but with a lower threshold value. The conductivities increased with more gold content and reached 10¹ S cm⁻¹ with weight fraction of gold up to 92 wt% (44.8 vol%).

More remarkably, the gold conducting layers had strong adhesion with the SNF membranes due to the compatibility with the SNFs in the gold conducting layer. The conducting pattern, with 74 wt% gold nanoplatelets as an example, demonstrated that there are strong binding forces between gold conducting layers and the SNF substrate to withstand the tearing of the tape (top image in Figure 6g). The transfer printing pattern was damaged by adhesive tape (bottom image in Figure 6g). The SEM image (Figure 6h) confirmed no gap was present between the gold conducting layer and the SNF membrane layer. In addition, these SNF membranes with connected nonporous structures can easily trap and transmit water molecules, and hence can be adhered to gloves and skins (Figure 6i and Figure S9, Supporting Information), and deformed with skins under 85% relative humidity (Figure 6j and Figure S9, Supporting Information), suggesting utility for electronic skins, biosensors, and microactuators.

In summary, we report a top-down approach to directly exfoliate SNFs from silk fibers. The exfoliated SNFs had diameters of 20 ± 5 nm with contour lengths of up to 500 nm, the same size as that of single nanofibers in native silk fibers, demonstrating the gentle nature of this new process to preserve native structures and features. This is the first method that extracts the SNFs from silk fibers at the single nanofibril scale. In addition, the SNFs displayed excellent structural and dispersive stability in water, providing the ability to fabricate useful macroscopic bio-nanomaterials. A vacuum-assisted filtration process was used with the SNF dispersions to generate well-structured membranes, which had excellent physical properties and could be further functioned for optical and electronic devices. These membranes show prospects for advanced materials toward tissue engineering, optical devices, nanoelectronics, and biosensors.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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