Supporting Information

Amorphous Silk Nanofiber Solutions for Fabricating Silk-Based Functional Materials

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Experimental Section

Dynamic light scattering (DLS)

Dynamic light scattering (DLS) measurement was performed with a Zetasizer (Nano ZS, Malvern, Worcestershire, UK) using an angle of 173° and equipped with a 633-nm He–Ne laser. 1ml of silk solution was used in disposable polystyrene cuvettes with a 10-mm path length. All data were collected at 25 ^{o}C.

Zeta Potential

Zeta potentials of silk solutions were recored by zeta potential measurement. For the measurement, one milliliter of the solution was loaded to a Zetasizer (Nano ZS, Malvern, Worcesteshire, UK) at 25 °C.

Atomic Force Microscopy (AFM)

For AFM experiments, SF-FA were diluted to below 0.001 wt% to avoid masking the original morphology by multilayers of silk.[1] A total of 2 μL of the diluted solution was dropped onto freshly cleaved 4 × 4 mm^2 mica surfaces and spin coating. The morphology of silk fibroin was observed by AFM (Nanoscope V, Veeco, NY, USA) in air. A 225 μm long silicon cantilever with a spring constant of 3 N m^{-1} was used in tapping mode at 0.5-1 Hz scan rate.

SEM

The morphology of samples was observed using scanning electron microscopy (SEM, S-4800, Hitachi, Tokyo, Japan) at 3kV. For the solution samples, the samples were diluted to below 0.001 wt% to avoid masking the original morphology, 2 ul of samples was added directly on top of a conductive tape mounted on a SEM sample

stub and dried in air. As for scaffolds and films, the samples were fractured in liquid nitrogen. Before SEM examination, all samples were coated with platinum for 60s.

Circular dichroism (CD)

The secondary structures of the silk solutions were collected using a Jasco-815 CD spectrophotometer (Jasco Co., Japan).[2] CD spectra were recorded from 250 to 190 nm wavelengths at a scanning rate of 100 nm min^{-1} with an accumulation of five scans at 25 ^{o}C. The results were averaged from three repeated experiments.

FTIR

FTIR was conducted on a Nicolet FTIR 5700 spectrometer (Thermo Scientific, FL, USA) equippedwith a MIRacle^{TM} attenuated total reflection (ATR) Ge crystal cell in reflection mode.[3, 4] For each measurement, 64 scans were coded with a resolution of 4 cm^{-1}, with the wavenumber ranging from 400 to 4000 cm^{-1}.

Raman Spectra

The secondary structures of the silk solutions were measured with a Raman spectrometer (Renishaw, 633 nm diode laser with a resolution of 2 cm^{-1}, exposure time 10s and laser power 100%) based on the freeze-dried samples.[5] The samples were placed at -20 ^{o}C for about 12 h, and then lyophilized for about 48 h to achieve freeze-dried samples.

DSC

Samples of about 5mg were encapsulated in aluminum pans and heated in a TA Instrument Q2000 DSC (TA Instruments, New Castle, DE, USA) with a dry nitrogen gas flow of 50 ml·min^{-1}. The instrument was calibrated for empty cell baseline and

with indium for heat flow and temperature before the test. Temperature-modulated differential scanning calorimetry (TMDSC) measurements were performed using a TA instrument Q2000, equipped with a refrigerated cooling system.[4] The samples were heated from -30 to 350^{o}C at 2^{o}C min^{-1} with temperature modulation amplitudes of 0.318^{o}C and a modulation period of 60s.

Dynamic oscillatory rheology

Rheological studies were run on a Rheometer (AR2000, TA Instruments, New Castle, USA). Prior to each experimental day, the rheometer underwent a torque map with a 10 Pa s calibration oil. The shear rate was linearly increased from 0.01 to 5000 s^{-1} at 25 ^{o}C (Ti, 40/2^{o}).[6] Frequency sweeps were collected continuously over a wide frequency range from 1 to 100 rad s^{-1} at 25 ^{o}C (Ti, 20/1^{o}).[7] All samples were stabilized for 20 min before the measurement.

Results

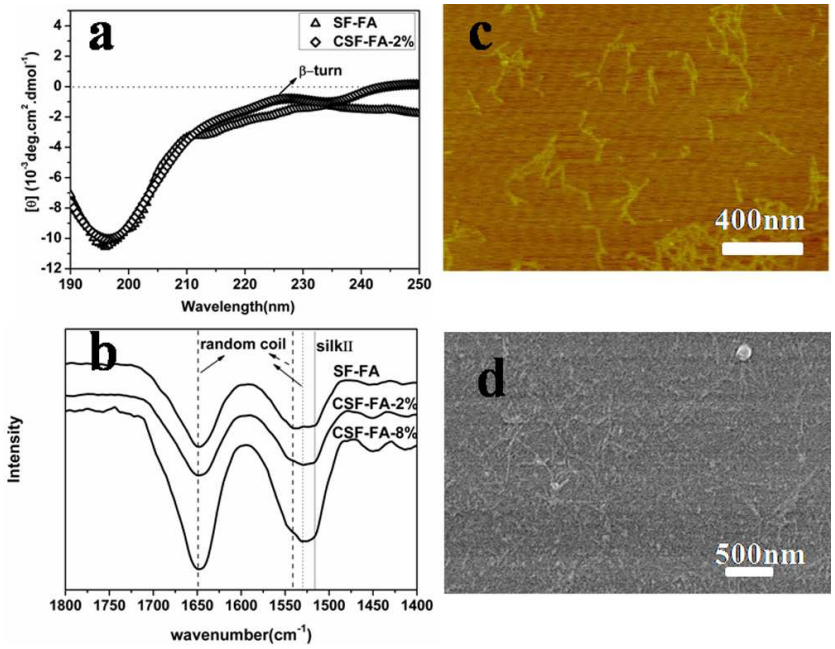

Figure S1. Conformations and microstructures of silk in the concentrated process: (a) CD; (b) FTIR of silk fibrion solutions. (c) AFM and (d) SEM of 2wt% silk nanofiber solutions. The samples are as follows: SF-FA, fresh amorphous silk nanofiber solution prepared through the present LiBr-formic solvent; CSF-FA-2%, fresh amorphous silk nanofiber solution were concentrated to 2wt%; CSF-FA-8%, fresh amorphous silk nanofiber solution were concentrated to 8wt%. No significant secondary structure and microstructure changes appeared after concentration.

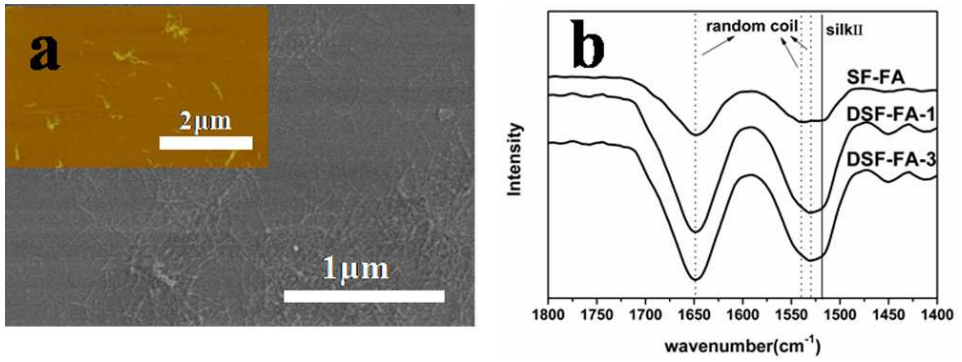

Figure S2.(a) SEM, AFM and (b) FTIR of re-dissolved silk nanofiber solutions. The samples are as follows: SF-FA, fresh amorphous silk nanofiber solution prepared through the present LiBr-formic solvent; DSF-FA-1, re-dissolved after silk nanofiber solutions had been freeze-dried, stored at room temperature for one month; DSF-FA-3, re-dissolved after silk nanofiber solutions had been freeze-dried, stored at room temperature for three months.

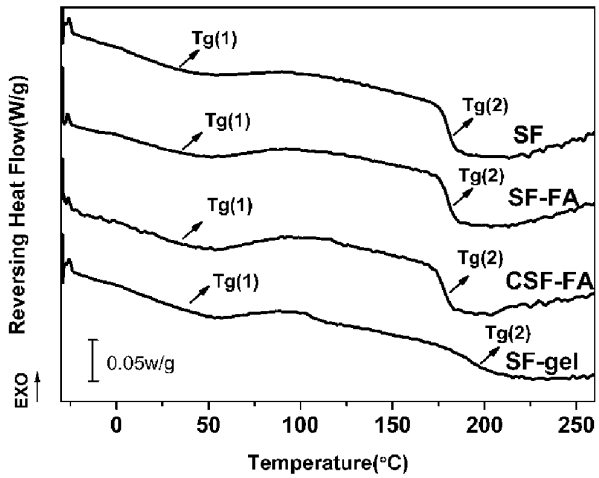

Figure S3. Temperature-modulated DSC scans (TMDSC) of different silk solutions. The samples are as follows: SF-FA, amorphous silk nanofiber solution prepared through the present LiBr-formic solvent; SF, amorphous silk solution prepared through the regular LiBr solvent reported previous; SF-gel, silk nanofibers with high beta-sheet content prepared through a slow concentration–dilution process; CSF-FA, fresh amorphous silk nanofiber solution were concentrated to 8%.

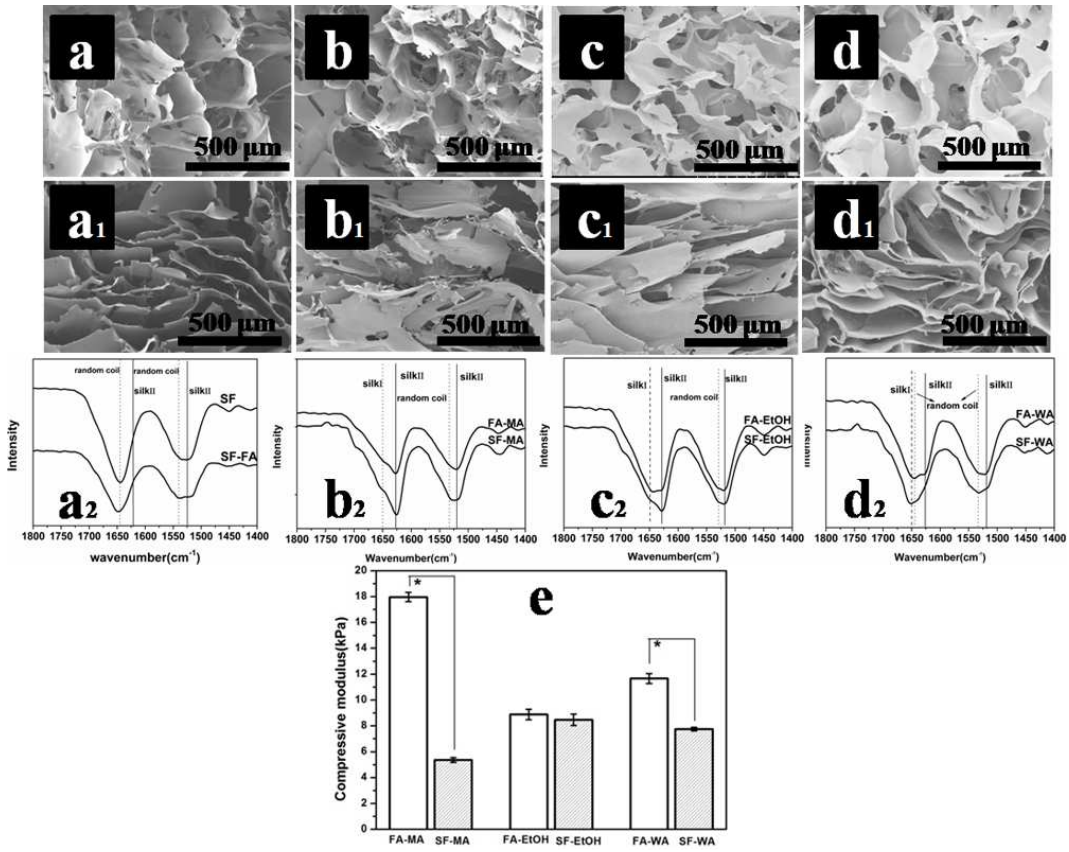

Figure S4. The properties of silk scaffolds derived from SF-FA and SF solutions: (a)-(d) SEM images of untreated methanol treated, ethanol treated and water annealing scaffolds derived from SF-FA, respectively; (a_1-d_1) SEM images of untreated , methanol treated, ethanol treated and water annealing scaffolds derived from SF, respectively; (a_2-d_2) The FTIR spectra of untreated methanol treated, ethanol treated, and water annealing scaffolds derived from SF-FA and SF solutions, respectively; (e) The mechanical properties of the scaffolds derived from SF-FA and SF, respectively. The methanol, ethanol and water annealing scaffolds were termed FA-MA, SF-MA, FA-EtOH, SF-EtOH, FA-WA, SF-WA, respectively. Error bars represent B mean ± standard deviation with N= 5 (*$p < 0.05$).

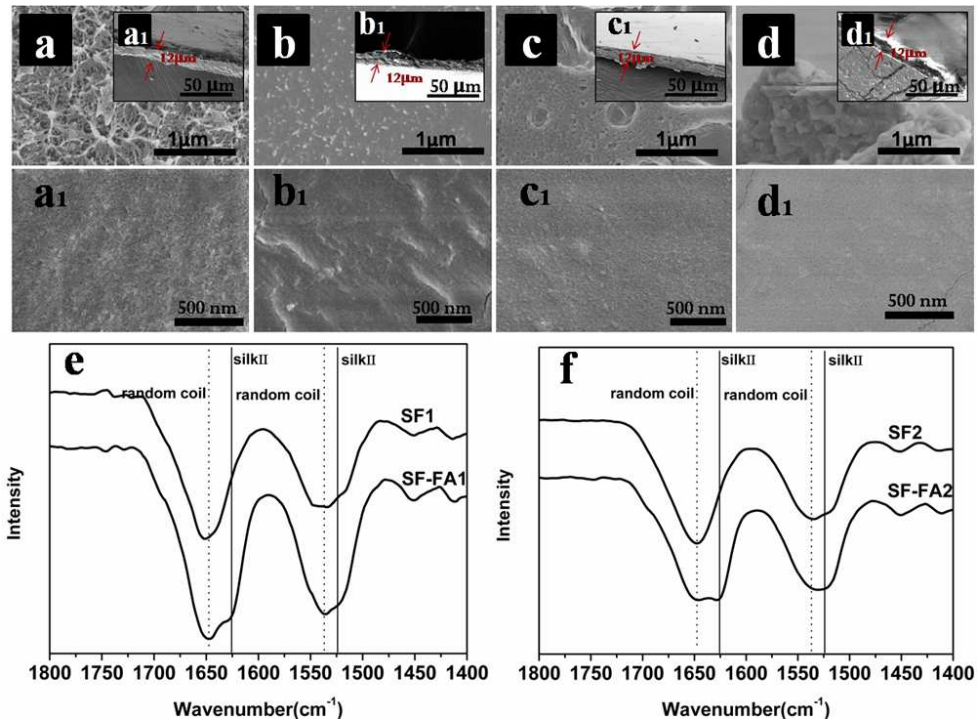

Figure S5. The properties of silk films derived from SF-FA and SF solutions: (a)-(d) Cross-section; (a_1)-(d_1) surface images; (a_2)-(d_2) thickness of films; (e) FTIR of films prepared by 2% silk solutions at room temperature. (f) FTIR of films prepared by 2% silk solutions at 60 ^{o}C. (a, a_1 and a_2) Drying the nanofiber solution overnight at room temperature in fume hood without lid; (b, b_1 and b_2) Drying the nanoparticle solution overnight at room temperature in fume hood without lid; (c, c_1, and c_2) Drying the nanofiber solution overnight at 60 ^{o}C in fume hood without lid; (d, d_1 and d_2) Drying the nanoparticle solution overnight at 60 ^{o}C in fume hood without lid.

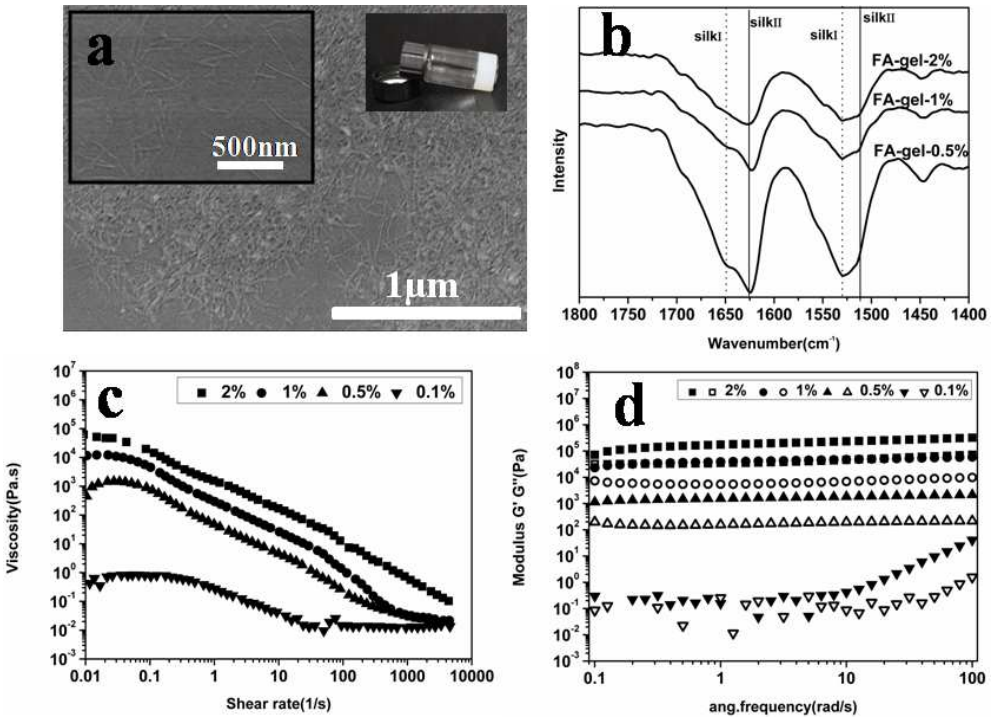

Figure S6. Silk hydrogels derived from the amorphous silk nanofibers by treating the solution at 60 ^{o}C for about 4 hours: (a) SEM images of the diluted silk hydrogels; (b) FTIR of the hydrogels derived from silk nanofiber solution with various concentrations; (c) Storage modulus (G', solid symbols) and loss modulus (G'', open symbols) versus frequency of the hydrogels with various concentrations. Compared to the previously reported SF-gel, SF-FA also formed hydrogels which were composed of nanofibers and have high crystallinity. The samples are as follows: FA-gel-2%, hydrogels from 2% amorphous silk nanofiber solutions placed at 60 ^{o}C for about 4 hours; FA-gel-1%, hydrogels from 1% amorphous silk nanofiber solutions placed at 60 ^{o}C for about 4 hours; FA-gel-0.5%, hydrogels from 0.5% amorphous silk nanofiber solutions placed at 60 ^{o}C for about 4 hours.

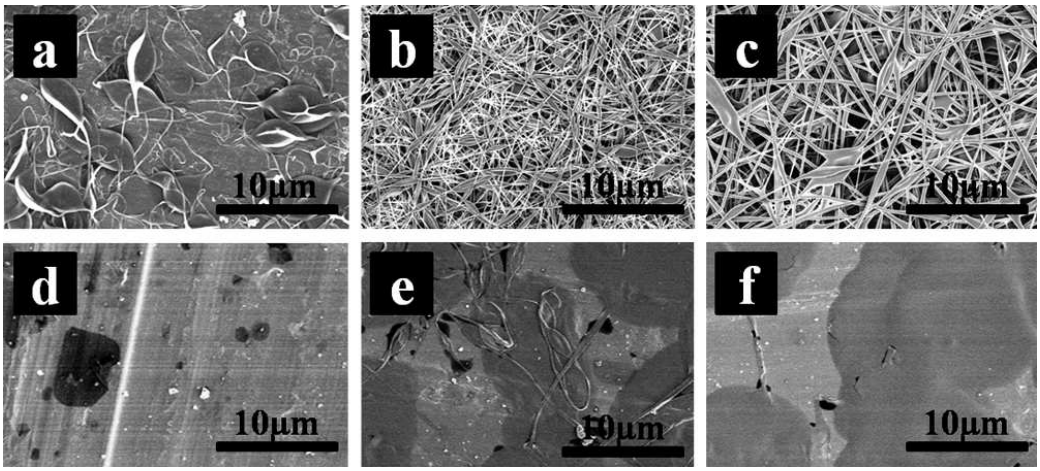

Figure S7. The morphology of electronspun silk nanofibers derived from SF-FA and SF solutions: (a)-(c) The SEM images of electorspun silk nanofibers from 2%, 6%, 8% SF-FA and (d)-(f) The failure to form electrospun silk nanofiber from 2%, 6%, 8% SF solution. The samples are as follows: Left, electorspun silk nanofibers from 2% silk solution; Middle, electorspun silk nanofibers from 6% silk solutions; Right, electorspun silk nanofibers from 8% silk solutions.

Table S1. Sample abbreviations of SF samples obtained under different conditions

Sample code	preparation method
SF-FA	amorphous silk nanofiber solution prepared through the present LiBr-formic solvent
SF	amorphous silk solution prepared through 9.3M LiBr solvent
SF-gel	silk nanofibers with high beta-sheet content prepared through a slow concentration–dilution process
CSF-FA	fresh amorphous silk nanofiber solution were concentrated to 8%
HSF-FA	silk hydrogels formed by SF-FA
HSF	silk hydrogels formed by SF

Table S2. Mechanical properties of silk films prepared by SF and SF-FA. n = 3, average ± standard deviation.

Silk Film	Tensile Strength (Mpa)	Elongation at Break (%)
SF-FA1	24.8±10.8	3.0±0.5
SF1	26.1±4.9	7.1±4.2
SF-FA2	29.1±13.5	3.8±0.5
SF2	26.6±5.5	6.8±5.1

Supporting Information References

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