

Supporting Information

**Thixotropic silk nanofibril-based hydrogel with
extracellular matrix-like structure**

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PREPARATION OF SF NANOFIBRILS AND HYDROGEL

Production of silk fibroin aqueous solution. The raw silk cocoon was degummed by boiling it twice in 0.5% (w/w) NaHCO_3 aqueous solution for 30 minutes. The degummed dry silk was then dissolved in 9.3 mol/L LiBr aqueous solution by heating to 60 °C for 1 h. Then the solution was dialyzed against deionized water for 72 h at room temperature with a 14,000 molecular weight cut off dialysis membrane to remove the salt and acquired the regenerated silk fibroin solution (about 5% (w/w)).

Production of SF nanofibrils. The silk fibroin solution was diluted to 0.75 wt%, and adjusted pH to 9.5 with 0.5 mol/L NaOH aqueous solution. Then ethanol was added (the final concentration 7 vol%). The mixture was incubated at room temperature for the appearance of SF nanofibrils.

Production of SF nanofibril-based hydrogel. Before producing hydrogels, SF nanofibril solution was dialyzed (3500 molecular weight cut off dialysis membrane) against deionized water for 3 days to remove the ethanol. Then 0.6 ml NaCl was added into 5.4 ml SF nanofibril solutions (final solutions with NaCl concentration of 25, 50, 75, 100 and 125 mmol/L). The solution was centrifuged for 10 min at 8000 r/min. Finally the upper water was removed.

CD CHARACTERIZATION

After removing salt and ethanol by dialyzing, SF nanofibril solution were analyzed with Jasco-715 CD spectrophotometer. CD spectra were recorded from 190 to 250 nm wavelengths with an accumulation of 5 scans at 25°C.

FTIR CHARACTERIZATION

Lyophilized SF nanofibril-based hydrogel was transferred to sample holders and analyzed with FTIR Spectrometer (Nicolet 6700) for secondary configuration.

IN VITRO DEGRADATION OF SF NANOFIBRIL-BASED HYDROGELS

Degradation of the SF nanofibril-based hydrogel samples were tested *in vitro*. Hydrogels were immersed in the PBS buffer solution (10 mmol/L) at 37 ± 1 °C, and samples were taken out to measure the mass loss. Weight remaining (W_R) was calculated according as $W_R = W_1/W_0 \times 100\%$, where W_0 and W_1 are the weights of the hydrogel before and after soaking, respectively.

DYNAMIC OSCILLATORY RHEOLOGY

All rheological tests were carried out at $37\pm 1^\circ\text{C}$ in strain controlled mode through a Physica MCR 301 rheometer (Anton Paar GmbH, Australia) operating in 25 mm parallel-plate configuration and 1 mm gap distance. 1 ml hydrogel was dispensed on the bottom plate. To minimize the water evaporation, low density mineral oil was around the hydrogel on the plate. Frequency sweeps were performed over a frequency range from 0.1 to 10 Hz (strain 0.1%). As for the shear recovery test, the hydrogel was firstly stimulated at a 0.1% strain which increased to 5000% for 6 seconds, and finally returned to 0.1%. The cycle was repeated for 7 times at least.

LIGHT TRANSMISSION CHARACTERIZATION

The optical transmittance of the SF nanofibril-based hydrogel (NaCl 125mmol/L) was measured in a custom-holder using UV-visible spectrophotometer (HITACHI U-2910) operating in a spectral range of 350-700 nm.

AFM, TEM AND SEM CHARACTERIZATION

Atomic force microscopy (AFM) was performed by using a Nanoscope VIII (Bruker, USA) in an intermittent tapping mode at a scan rate of 1 Hz by using aluminum silicon cantilevers with a typical tip radius below 10 nm (Bruker, USA). A 20 μL aliquot of solutions was deposited onto freshly cleaved mica and incubated for 2. Then the samples were rinsed with Milli-Q water and gently dried in air before measurement.

Transmission electron microscopy (TEM) was performed by a Philips TEM (CM 20) instrument operating at a voltage of 100 kV. A drop of the SF nanofibril solution was casted onto a carbon support film on a copper grid. The excess solution was removed after 30 s by blotting using a filter paper.

Scanning electron microscopy (SEM) measurements were performed on HITACHI S-4800 operated at 1 kV. Images were collected on sections produced after freeze-fracture.

ZETA POTENTIAL CHARACTERIZATION

After removing the ethanol and NaOH, 0.7 ml SF nanofibril solution was loaded to Zetasizer (Malvern ZS90) for the zeta potential measurement at 25 $^\circ\text{C}$

CYTOTOXICITY TEST

Biocompatibility evaluation of SF nanofibril-based hydrogels was carried out by test the extraction medium using L929 cells according to the ISO standard (ISO10993.12-2004). The hydrogels were sterilized by soaking in ethanol aqueous solution (75% (v/v)) and washed to remove ethanol. Then the hydrogels were placed into a culture plate with fresh culture medium (Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin) added at $1.25 \text{ cm}^2/\text{mL}$ under sterilized conditions. The L929 cells were cultured at a density of $2.0 \times 10^4 \text{ cells/mL}$ on 96-well plates (with 200 µL per well) in a CO₂ (5%) incubator at 37 °C. After incubation for 24 h, the extracts of the hydrogels were added to replace the culture medium (with 200 µL per well) of the cells. In the meantime, the fresh culture medium was added to the control group. After incubation for 24 and 72 h, the culture medium was replaced with fresh serum-free DMEM. Then, CCK-8 was added in the dark, and the absorbance of the solution at 450 nm was examined using an Elx 800 instrument (Biotek, U.S.A.) after incubation for 2 h. The relative cell viability (%) was calculated as follows:

$$\text{Cell viability (\%)} = [A]_{\text{test}}/[A]_{\text{control}} \times 100\%$$

where $[A]_{\text{test}}$ is the absorbance of the absorbance of the test sample, and $[A]_{\text{control}}$ stands for the absorbance of the control sample.

CELL ENCAPSULATION IN SITU

The cell viability in the hydrogel was imaged by a confocal laser scanning microscope (Zeiss Ism710, Germany) after use of a live/dead staining kit. **100 µL cell suspension was added and mixed with the hydrogel to reach a final concentration of $1.0 \times 10^6/\text{mL}$.** Then injected hydrogels with L929 cells were incubated in culture medium (Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin) at 37 °C in an incubator containing 5% CO₂ for scheduled time, and live/dead imaging test was followed.

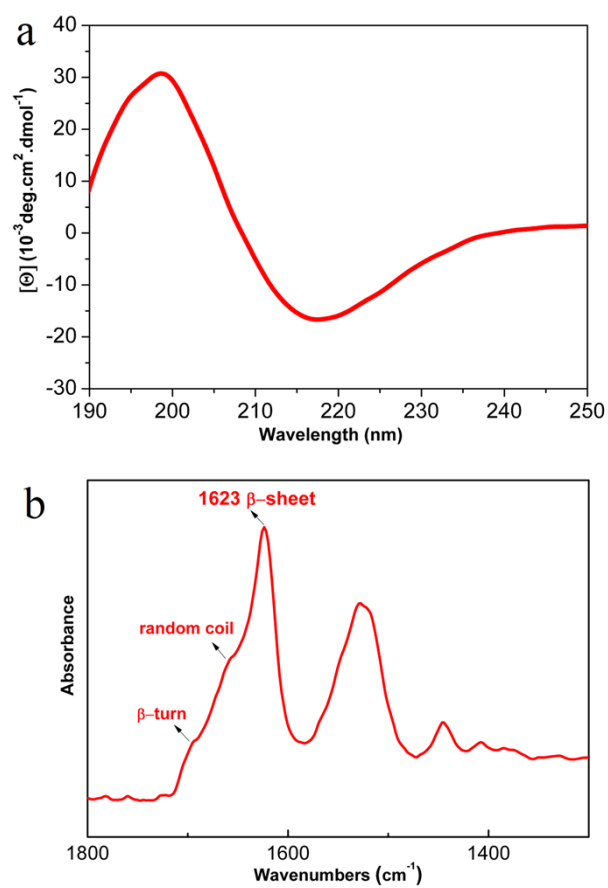


Fig. S1 Structural characterization of SF nanofibrils. a, CD spectrum of SF nanofibril solution. b, FTIR spectrum of lyophilized SF nanofibrils.

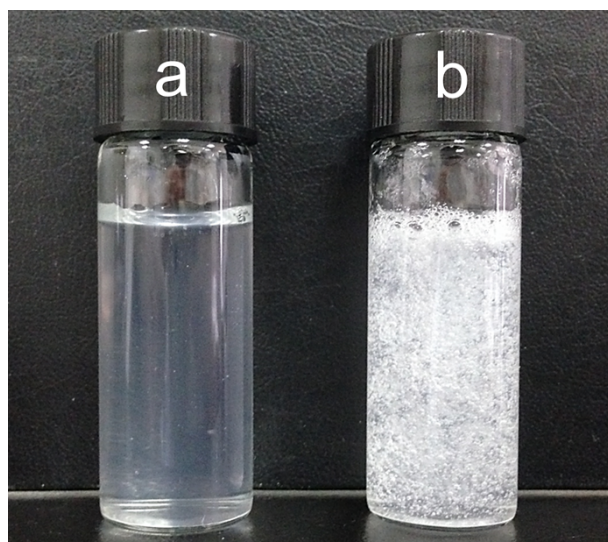


Fig. S2 The effect of adding NaCl into SF nanofibril solution. a, Vibrated SF nanofibril solution before adding 25 mmol/L NaCl, which is transparent with opalescent. b, Vibrated SF nanofibril solution after adding 25 mmol/L NaCl, which has many white bubbles, indicating NaCl undermined the stability of SF nanofibrils.

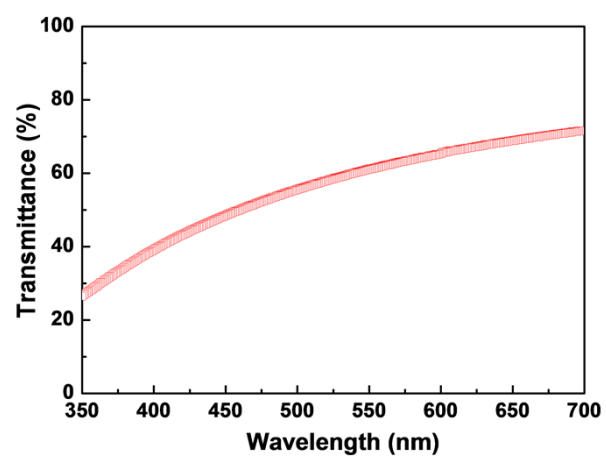


Fig. S3 Optical transmittance of SF nanofibril-based hydrogel.

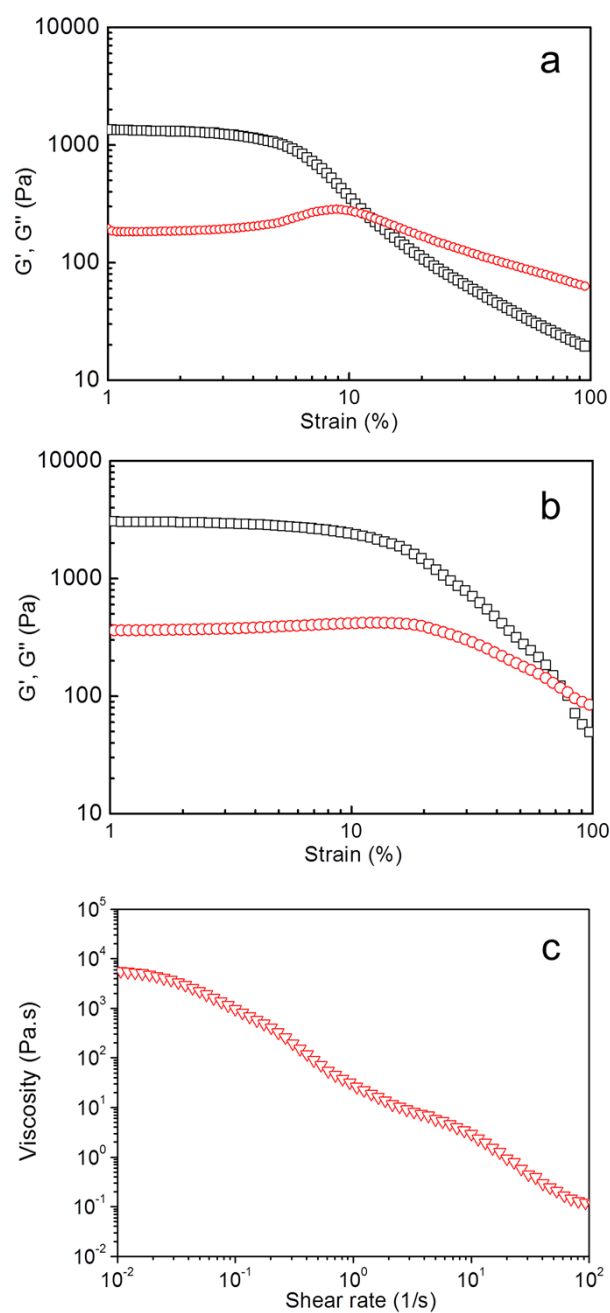


Fig. S4 Rheological characterization of SF nanofibril-based hydrogels. a, b Storage modulus (G') versus strain for SF nanofibril-based hydrogels with 25 and 125 mmol/L NaCl at 37 °C. c, Apparent viscosity verse shear rate for SF nanofibril-based hydrogel with 125 mM NaCl at 37 °C.

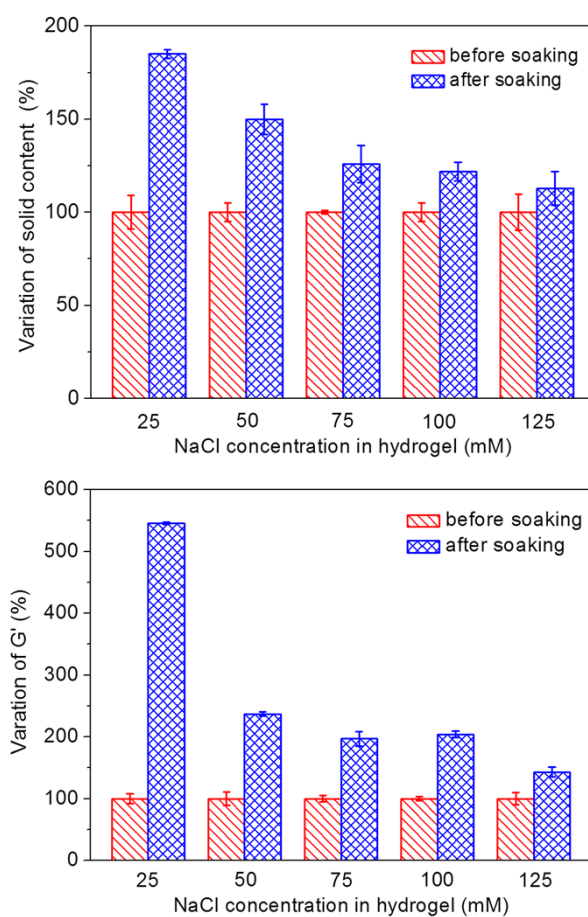


Fig. S5 Variation of SF nanofibril-based hydrogels soaked in PBS (150 mM NaCl) for 24 hours at 37 °C. a, Variation of solid content after being soaked in PBS. b, Variation of G' after being soaked in PBS.

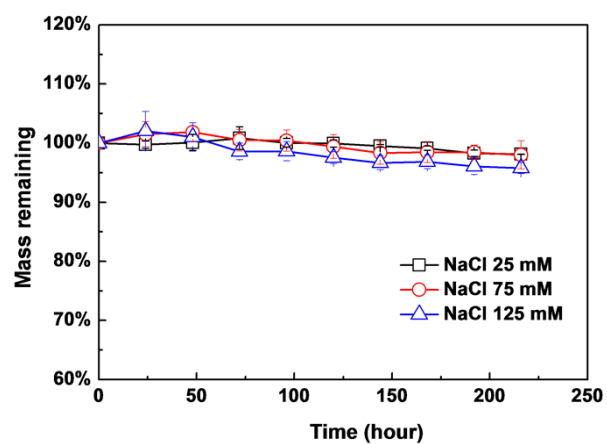


Fig. S6 *In vitro* degradation of the SF nanofibril-based hydrogels in 0.01 mol/L PBS with initial pH 7.4 at 37 °C.

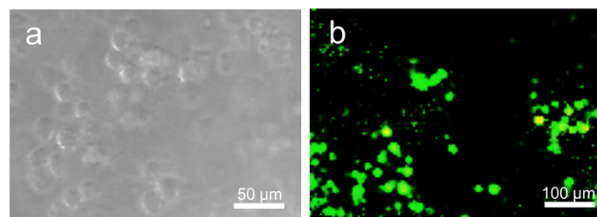


Fig. S7 The microphotograph (a) and fluorescent image (b) of L929 cells encapsulated within SF nanofibril-based hydrogel after 7 days culturing time.