Supporting Information

Reversible hydrogel-solution system of silk with high beta-sheet content

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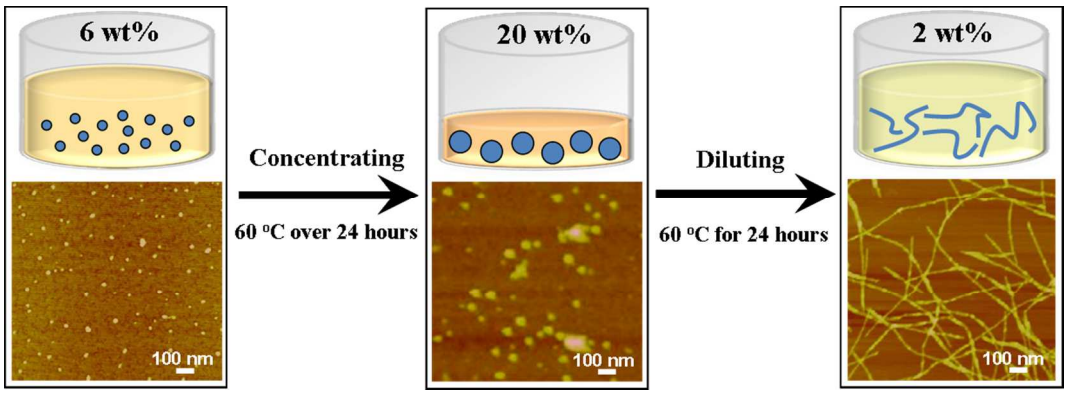

Figure S1. The forming process of silk flowing hydrogel composed of nanofibers. Fresh silk fibroin solution was slowly concentrated to 20% following metastable bigger nanoparticle formation, and then diluted to below 2% and cultured for 24 hours at 60 ^{o}C for inducing the nanofiber assembly.

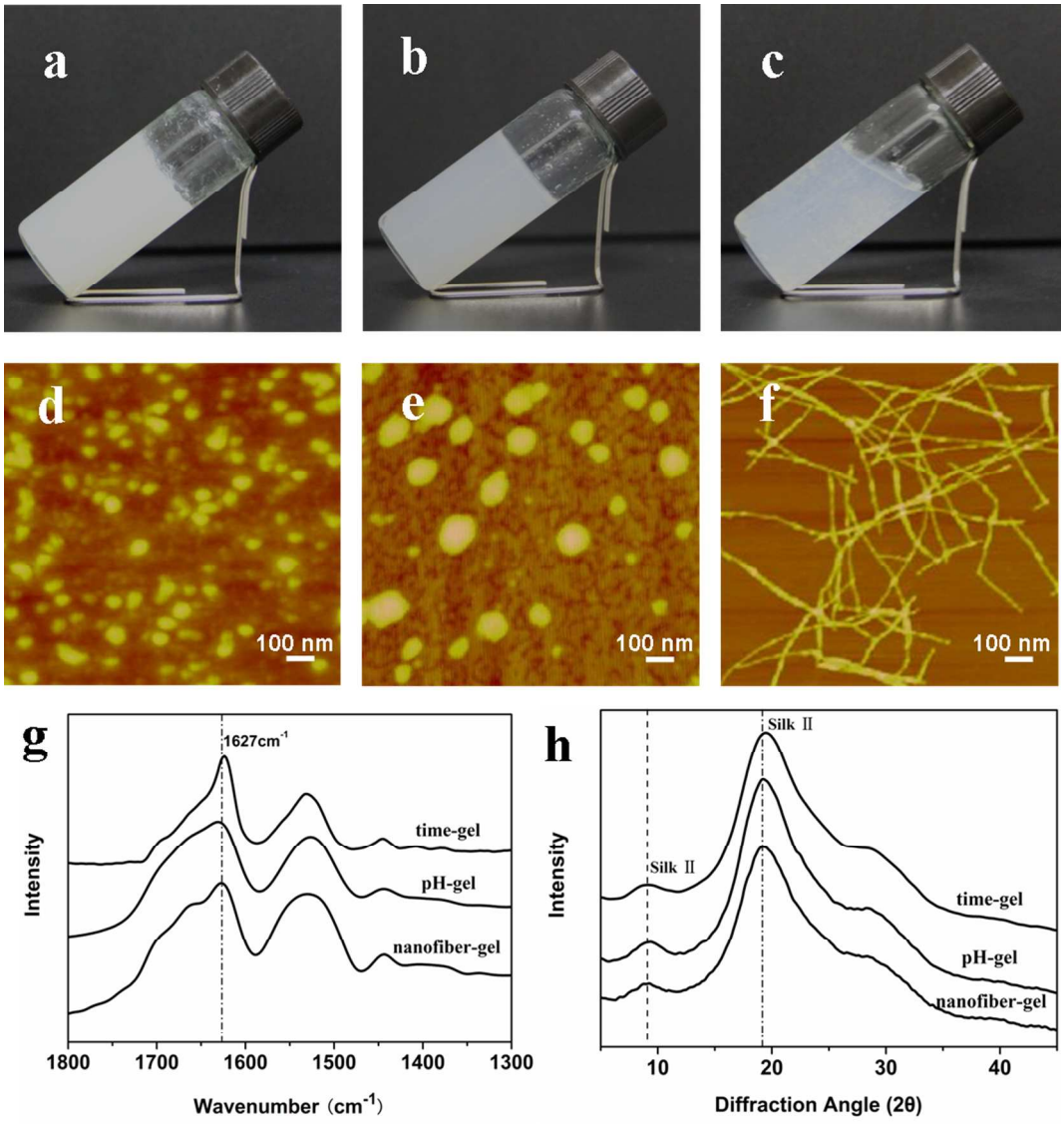

Figure S2. The difference of flowability (a-c) and nanostructures (d-f) in silk hydrogels prepared through various ways and the secondary structures of the different gels (FTIR spectra (g) and XRD peak (h)). The gels were prepared by culturing fresh solution for 1 month at 60 ^{o}C (a, d, time-gel), adjusting pH to isoelectric point of silk and then culturing the solution for 3 days at 60 ^{o}C (b, e, pH-gel), and slowly concentrating-diluting-culturing for 24 h at 60 ^{o}C (c, f, nanofiber-gel), respectively. The silk concentration was 2% in the different hydrogels.

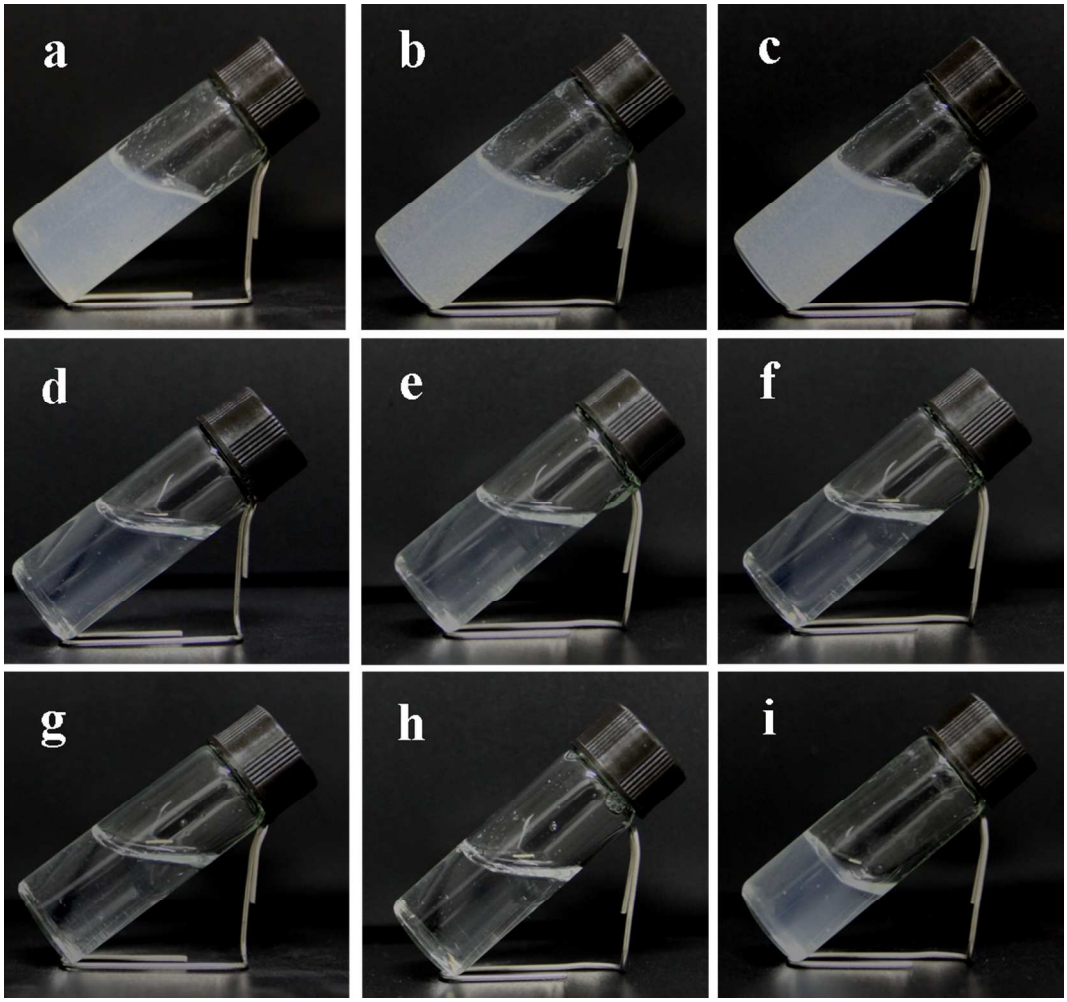

Figure S3. The stability of the flowing hydrogels and solutions composed of silk nanofibers. The flowing silk nanofiber hydrogels (2%, a) and nanofiber solutions (0.1%, d) maintained their flowing ability (b, c) and solution state (e, f) when cultured for 30 days at 4 ^{o}C and 90 ^{o}C, respectively, implying their good stability. The fresh silk solution (0.1%, g) kept solution state when cultured at 4 ^{o}C for 30 days (h), but transformed into hydrogel when placed at 90 ^{o}C for 30 days (i).

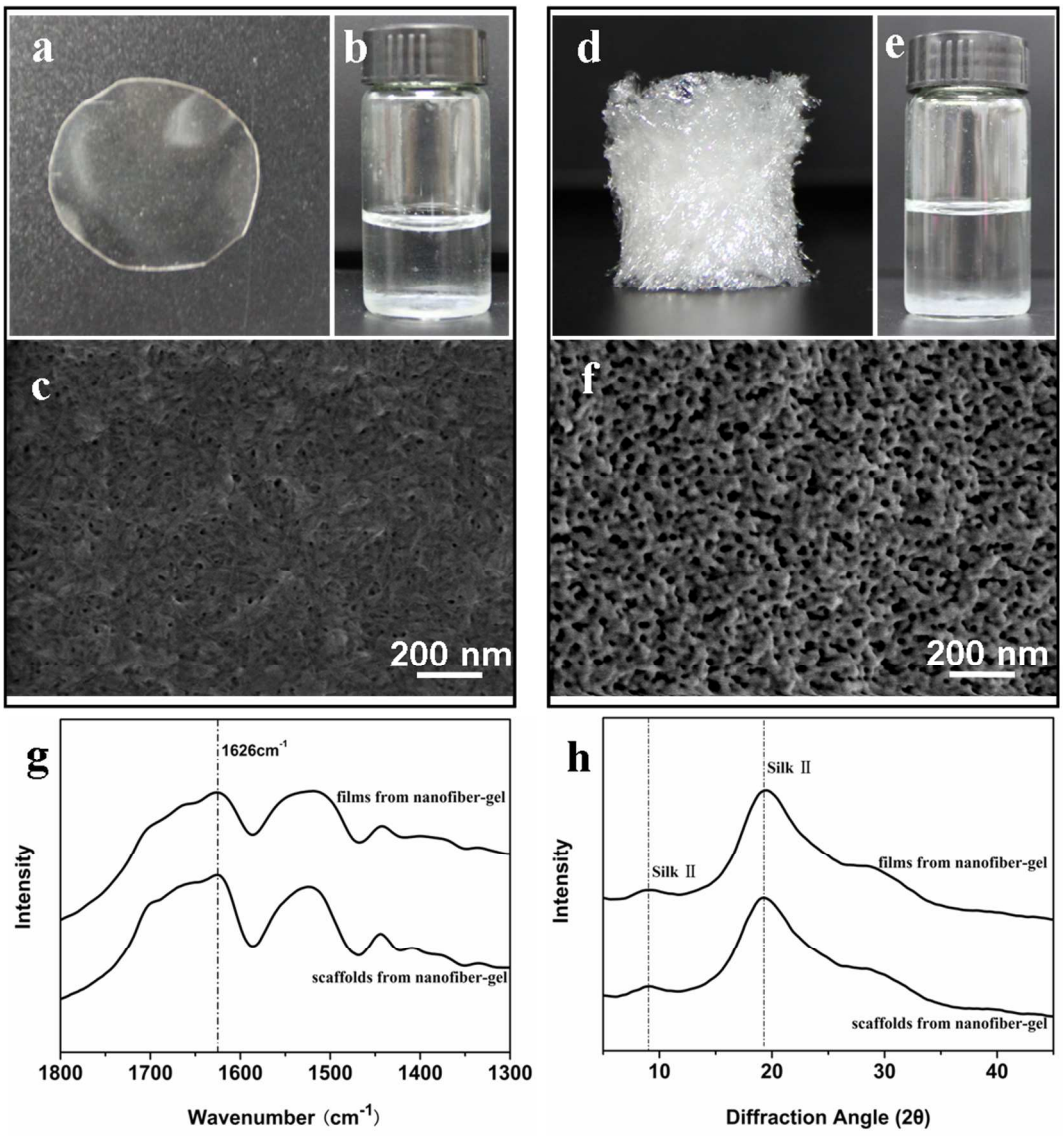

Figure S4. Silk films (a) and scaffolds (d) derived from the nanofiber hydrogel. Although the films and scaffolds maintained nanofiber structures (c, f) and high β-sheet content ((FTIR spectra (g) and XRD peak (h))), both the films and scaffolds were easily re-dissolved in water (b, e), different to previous water-insoluble silk films and scaffolds having similar β-sheet content.

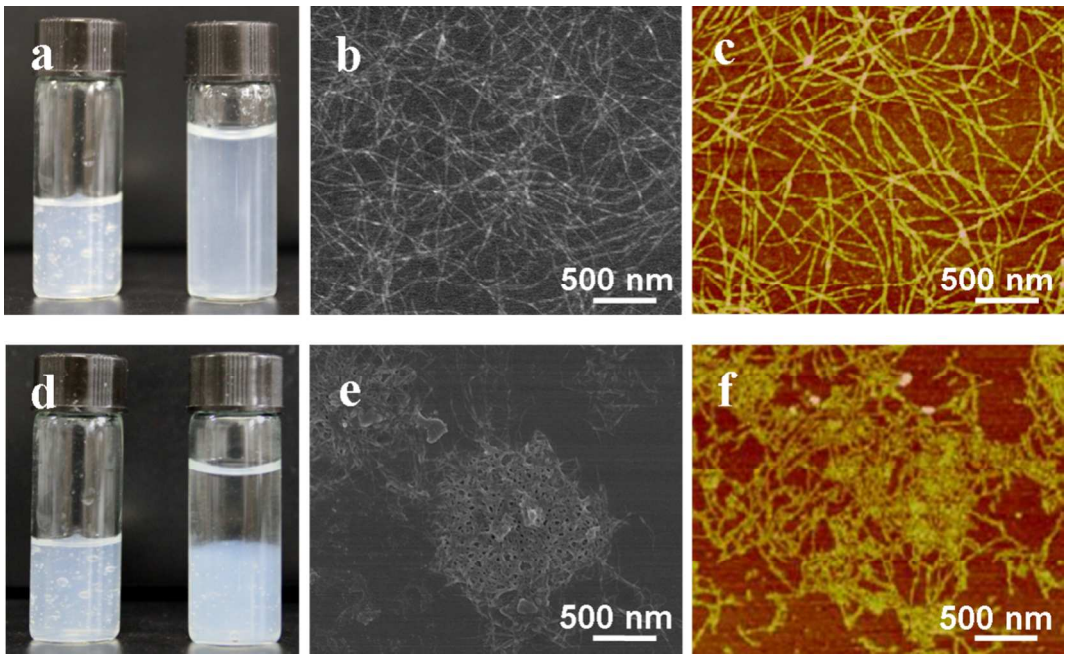

Figure S5. The reversibility control of the silk nanofiber system with calcium chloride. The silk nanofiber hydrogel without calcium chloride showed high Zeta potential to restrain the aggregation of the nanofiber (a-c). Calcium chloride could be added into silk nanofiber hydrogels to neutralize the negative charge, resulting in the aggregation of nanofibers and the loss of the reversibility (d-f).

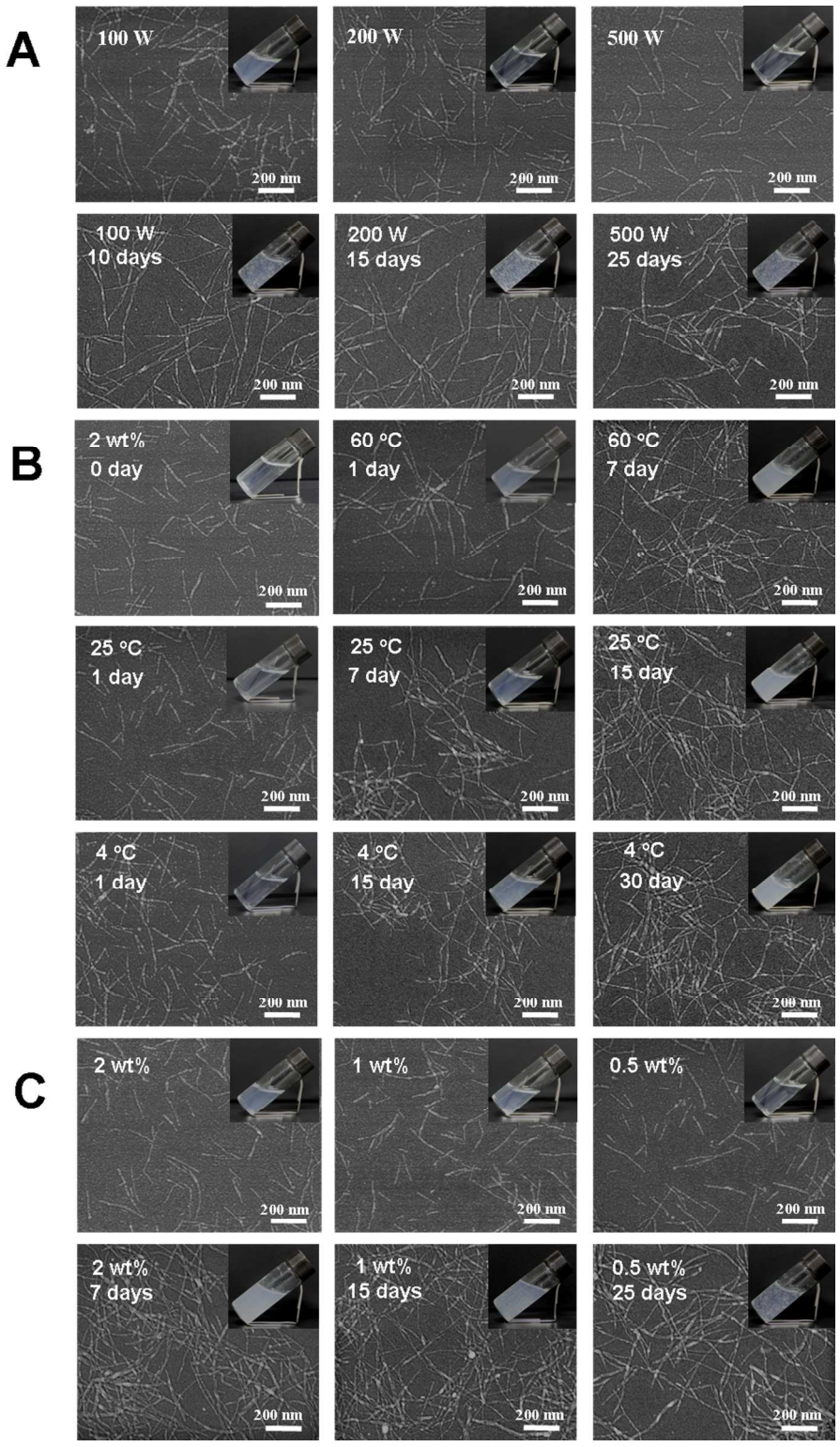

Figure S6. The solution-hydrogel transition under different conditions in ultrasonic treatment-culturing processes. (A) Silk solutions (0.5%) composed of nanofibers with different lengths

were prepared by changing the intensity of ultrasonic treatments. Then these silk fibers with different lengths were cultured at 60 ^{o}C until hydrogel formation. The solutions with longer nanofibers achieved faster solution-hydrogel transition rate; (B) The solution-hydrogel transition of silk nanofiber solution (2 wt%) prepared by ultrasonic-treating silk nanofiber hydrogel for 10 min with the intensity of 500 W. The regrowth rate of nanofiber as well as hydrogel transition rate increased at higher temperature. Silk hydrogels composed of nanofibers above 1 μm in length formed after 7 days, 15 days and 30 days when the culture temperatures were 60 ^{o}C, 25 ^{o}C and 4 ^{o}C, respectively; (C) The solution-hydrogel transition of ultrasonic treated silk when the silk solution with different concentrations cultured at 60 ^{o}C. The silk solutions were prepared by ultrasonic-treating silk nanofiber hydrogel (2 wt%) for 10 min with the intensity of 500 W and then diluted to 1 wt% and 0.5 wt%, respectively. The re-growth rate increased following the increase of silk concentration, resulting in faster transition from solution to hydrogel at higher concentration.