Supporting Information


Mechanically Robust Shape-Memory Organohydrogels Based on Silk Fibroin with Organogel Microinclusions of Various Sizes

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Experimental

Materials. Bombyx Mori silkworm cocoons were obtained from Kozabirlik (Agriculture Sales Cooperative for Silk Cocoon, Bursa, Turkey). Lithium bromide (LiBr, Merck, 99%), Na$_2$CO$_3$ (Merck, 99.9%), n-octadecyl acrylate (C18A, Sigma-Aldrich, 97%), N,N'-methylenebis(acrylamide) (BAAm, Sigma-Aldrich, 99%), 1,4-butanediol diglycidyl ether (BDDE, Sigma-Aldrich), N,N,N',N'-tetramethylethylenediamine (TEMED, Sigma-Aldrich), $\alpha,\alpha'$-azoisobutyronitrile (AIBN, Merck), ethanol (Merck, $\geq$ 99.9), and polyethylene glycol (PEG-10000, Sigma-Aldrich, 10,000 g.mol$^{-1}$) were used as received. Acrylic acid (AAc, Merck) was purified from its hydroquinone inhibitor by filtering through an Al$_2$O$_3$ column. Silk fibroin (SF) was isolated from Bombyx mori cocoons as previously reported [1]. Briefly, around 10 g of Bombyx mori silkworm cocoons were cut into small pieces and, after cleaning with distilled water, they were boiled in an aqueous solution of 0.02 M Na$_2$CO$_3$ (1 L) for 1 h to remove sericin proteins.
After five-time washing of the SF with 1 L of distilled water at 70 °C for 20 min each, it was dried at 23±2 °C for 2 days. 7 g of dried SF was then dissolved in 9.3 M LiBr solution (35 mL) at 60 °C within 2 h. The SF solution was then dialyzed using a 10,000 MWCO dialysis tubing (Snake Skin, Pierce) for 3 days against water that was changed three times a day. After centrifugation, the final SF concentration was ~5 wt. %, which was determined by weighing the remaining solid after drying. The aqueous SF solutions of higher concentrations were prepared by dialysis of 5 wt. % SF against aqueous 15 w/v% PEG-10000 solution using 3,500 MWCO dialysis tubing (Snake Skin, Pierce). All SF solutions were stored at 4 °C and used within 2 weeks.

**Synthesis of Cryogels and Organohydrogels.** SF cryogels were prepared from aqueous SF solutions of four different SF concentrations ($C_{SF}$), namely 5, 10, 15 and 20 w/v %. TEMED was used as a catalyst and pH regulator at a fixed amount of 0.025 v/v % with respect to the reaction volume [1,2]. The concentration of BDDE cross-linker was selected as 20, 10, 10, and 3 mmol per gram of SF for $C_{SF} = 5, 10, 15, \text{ and } 20$ w/v %, respectively, in order to prevent the undesired instant gelation at high SF concentrations. The typical procedure for the preparation of cryogels with 5 w/v % SF is as follows: 3.8 mL of a 13.2 wt % SF stock solution was mixed with TEMED (25 µL) and BDDE (1.94 mL). After completing the total volume to 10 mL with distilled water, the solution was transferred to plastic syringes of 1 mL in volume. The syringes were then placed in a refrigerator at -18 °C for 24 h to conduct the cryogelation reactions. After thawing the frozen cryogels at room temperature (23±2 °C), they were immersed in pure water for 7 days by refreshing water every day to extract the unreacted and soluble components. The water swollen cryogels were then freeze-dried in a Christ Alpha 2-4 LD-plus lyophilisator (Germany).

For the preparation of OHGs, freeze-dried cryogels formed at various $C_{SF}$ were immersed in ethanolic organogel precursor solutions at 40 °C containing AAc, C18A, BAAm and AIBN. The total monomer concentration was fixed at 41 w/v %. AIBN and BAAm amounts were also kept constant at 1 mol % with respect to the monomers. In order to investigate the effect of C18A on the crystallinity of OHGs, C18A mole fraction ($x_{C18A}$) in the monomer mixture was taken as 0.10, 0.20, 0.25 and 0.30. Typically, to prepare OHGs with $C_{SF} = 20$ w/v % and $x_{C18A} = 0.30$, an ethanolic organogel precursor solution was first prepared by mixing C18A (2.7 g), AAc (1.32 mL), BAAm (42.8 mg), and AIBN (45.6 mg), and diluting to 10 mL total volume with ethanol. After immersing the freeze-dried cryogel specimens in the organogel precursor solution for 10 min, they were placed
into 10 mL plastic syringes along with the remaining organogel precursor solutions. Syringes were then kept in an oven at 50 °C for 24 h to conduct the free radical copolymerization reactions. After polymerization, OHGs formed were separated from the surrounding excessive organogel layer (Figure S5), and kept in ethanol for at least one week to extract the soluble species. The OHGs were then transferred into water for two weeks during which water was refreshed several times. For comparison, poly(AAc-co-C18A) organogels with $x_{C18A} = 0.30$ were also synthesized under the same conditions but in the absence of SF cryogels.

**Characterization.** The weight ($q_w$) and volume ($q_v$) swelling ratios of the cryogels and OHGs with respect to their dry states were calculated using the equations,

\[ q_w = \frac{m}{m_{dry}} \quad \text{(S1a)} \]

\[ q_v = \left(\frac{D}{D_{dry}}\right)^3 \quad \text{(S1b)} \]

where $m$ and $D$ are the mass and the diameter of the swollen specimens, respectively, and $m_{dry}$ and $D_{dry}$ have the same meanings but for their dry states. Moreover, the equilibrium mass ($m_{rel}$) and volume ($V_{rel}$) swelling ratios with respect to the as-prepared state were calculated by

\[ m_{rel} = \frac{m}{m_o} \quad \text{(S2a)} \]

\[ V_{rel} = \left(\frac{D}{D_o}\right)^3 \quad \text{(S2b)} \]

where $m_o$ and $D_o$ are the mass and the diameter of the as-prepared specimens, respectively. The gel fraction ($W_g$) was calculated by,

\[ W_g = \frac{m_{dry}}{C_o m_o} \quad \text{(S3)} \]
where \( C_0 \) is the total concentration of the reactants. The total volume of the pores \( V_p \) was estimated from the mass increase of the scaffold and dried OHGs in water at pH = 3.0. It was found that in this solution, both the scaffold and OHGs do not swell by volume, i.e., \( q_v = 1 \), so that the mass increase is related to the amount of solvent within the pores. \( V_p \) was calculated as

\[
V_p = \frac{(m_p/m_{dry}) - 1}{d_1}
\]  

where \( m_p \) is the mass of the specimen in water at pH = 3.0, and \( d_1 \) is the density of water taken as 1.0 g·mL\(^{-1}\). Conformational analysis of SF cryogels was performed with the Agilent Technologies Cary 630 ATR-FTIR spectrophotometer as reported previously [1,2]. X-ray diffraction (XRD) measurements were conducted on a Rigaku Ultima-IV Diffractometer using Ni-filtered CuK\(\alpha\) radiation at 40 kV and 30 mA in the range of 20 = 4 - 40\(^\circ\) with a scanning rate of 0.6\(^\circ\)/min. Differential scanning calorimetry (DSC) measurements were performed on a Perkin Elmer DSC 4000 instrument under a nitrogen atmosphere. Water-swollen cryogel and OHG samples each weighing around 10 mg were placed in aluminum pans of the instrument, and subjected to two heating-cooling cycles between 0 – 80 \(^\circ\)C to determine the phase transition temperatures, melting enthalpies and crystallization degrees (\( f_{cry} \)) as previously reported [3,4]. Scanning electron microscopy (SEM) imaging was performed using platinum-coated samples with an FEI-QUANTA FEG 250 FE-SEM peripheral electron microscope. The pore size distribution analysis was performed by measuring the diameter of 50 randomly selected pores from the SEM images using the Image-Pro Plus 6 software and creating density distribution curves from the obtained data.

Uniaxial compression tests were carried out at 23±2 \(^\circ\)C on a Zwick Roell instrument using a 500 N load cell at a compression speed of 5 mm·min\(^{-1}\). The load and deformation data are presented as the dependence of the nominal stress \( \sigma_{nom} \) (force acting per cross-sectional area of the undeformed specimen) on the strain \( \varepsilon \) (change in the length / initial length of the specimen). Young's modulus \( E \) was calculated from the slope of \( \sigma_{nom} - \varepsilon \) curves between 2 – 4\% strains. The fracture stress \( \sigma_f \)
and strain $\epsilon_f$ were calculated from the maxima of the true stress – strain curves as detailed before [5].

Rheological tests were carried out on a Bohlin Gemini 150 rheometer instrument equipped with a parallel-plate geometry with a diameter of 20 mm, and a Peltier device for temperature control. During all measurements, a solvent trap was used to minimize the evaporation. The frequency sweep tests were performed at a constant strain magnitude of 0.1% between 1-60 Hz frequencies. The shape memory tests were performed using the bending test [6]. The cylindrical OHG specimen of 5 cm in length and 4 mm in diameter was heated to 65 °C and deformed into a horseshoe-like shape to create a certain deformation angle ($\theta_d$) by bending its two ends. The specimen was then kept at 25 °C for 5 min to fix the temporary shape, and the fixed deformation angle ($\theta_f$) was measured. The OHG specimen was then reheated to 70 °C during which the recovery angle ($\theta_r$) in each 1–3 °C temperature intervals were measured. The shape fixity ($R_f$) and recovery ratios ($R_r$) were determined by,

$$R_f = \frac{\theta_f - 180}{\theta_d - 180} \quad (S5)$$

$$R_r = \frac{\theta_d - \theta_r}{\theta_d - 180} \quad (S6)$$

**Calculation of swollen state porosity**

To estimate the porosity of the cryogels and OHGs in their swollen states, we used their weight ($q_w$) and volume swelling ratios ($q_v$) in water. We should note that the $q_w$ of cryogels includes water locating both in the gel and in the pores while $q_v$ is caused by the solvation of the cross-linked polymer chains, i.e., pore walls. Thus, the swollen state porosity $P_{sw}$ of the cryogels and OHGs can be calculated from the difference between $q_w$ and $q_v$ using the equation [7],

$$P_{sw} = 1 - \frac{q_v}{1 + (q_w - 1) \rho/d_1} \quad (S7)$$
where $d_1$ and $\rho$ are the densities of water and SF scaffold or OHG, respectively. Assuming equals densities for water and polymer, eq. S7 reduces to eq. (1) in the text.

References


Figure S1. Gel fraction $W_g$ of SF cryogels plotted against $C_{SF}$. 

S6
**Figure S2.** FTIR spectra of freeze-dried SF cryogels prepared at various $C_{SF}$ as indicated. The original data are shown by the filled circles while solid and dashed curves are the results of curve fitting for the original spectrum and hidden peaks, respectively.

**Figure S3.** Pore wall thickness of cryogels plotted against $C_{SF}$. 

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Figure S4. Swollen state porosity $P_{sw}$ of cryogels (open symbols) and OHGs (filled symbols) plotted against $C_{SF}$.

Figure S5. Images showing the cylindrical SF scaffolds immersed in an organogel precursor solution (a), and the resulting OHG after free radical polymerization (b). The OHG specimens and surrounding organogel in (b) appear white and transparent, respectively.