Supporting Information

Freezing-triggered gelation of quaternized chitosan reinforced with microfibrillated cellulose for highly efficient removal of bilirubin

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Experiment section

DQ measurement of QCS: The *DQ* of chitosan derivatives were quantitatively determined from the ^{1}H NMR spectrum according to the ratio of integral of hydrogen atom bonded to Carbon 1 of chitosan backbone and hydrogen atoms of modified active moieties. For instance, the *DQ* of QCS was carried out from the following equation:

$$DQ(\%) = \frac{\frac{I_{H_d,QCS}}{9}}{I_{H_1,QCS}} * 100 \quad (1)$$

Where $I_{\mathrm{Hd,\ QCS}}$ is the integral of trimethyl ammonium protons of $N^{+}(CH_3)_3$ group (H_d, 3.12 ppm) of QCS, 9 means the number of protons in H_d of QCS. H_1 is used as an integral standard peak, and $I_{\mathrm{H1,\ QCS}}$ is the integral of hydrogen atom bonded to Carbon 1 of chitosan backbone (4.43 ppm).

MTT assay: MTT assay was performed to evaluate the cytotoxicity of the QCS/MFC cryogel toward fibroblasts. A certain amount of sample material is weighed and placed in a 15 mL centrifuge tube and sterilized at 121 °C for 30 min. The sterilized cryogels were immersed in dulbecco's modified eagle medium (DMEM) and extracted at 37 °C for 72 h, followed by centrifugation at 1000 rpm for 10 min to obtain extract solution. L929 cells were cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution and seeded in 96-well plates with a density of 1×10^4 cells per well, 200 μL per hole. When the cells adhered and grew normally, the original medium was discarded and replaced by the medium containing 50 μL of QCS/MFC extract. The medium with the same concentration and no extract was used as the

negative control group. After incubation for 24, 48, and 72 h, respectively, 20 μL of 5 mg/mL MTT solution was added to each well and incubated at 37 °C for another 4 h. Then the medium was carefully discarded and 200 μL of DMSO was added to each well. The optical density of each well at 490 nm was detected by ELISA reader. All the tests were repeated three times and the mean calculated value was employed as the final result. The cell viability (%) normalized by the control and was measured according to:

$$\text{Cell viability}\ (\%) = \frac{A_S}{A_{control}} \times 100 \quad (2)$$

Where A_S and $A_{control}$ are the absorbance values of the sample and the control sample respectively.

Hemolysis Evaluation: Fresh anticoagulant whole blood was collected and then diluted with physiological saline at a ratio of 1:1.25. The samples were cut into discs with a thickness of 0.5 cm, placed in a 15 mL centrifugal tube, washed with distilled water 3 times, rinsed in 0.9 wt% NaCl solution for 30 min. After pouring out the soaking solution, 10 mL normal saline was added to incubate in a water bath at 37 °C for 30 min, followed by the addition of 0.2 mL of diluted whole blood to continuously incubate in a water bath at 37 °C for 60 min. Subsequently, all samples were centrifuged at 1500 rpm for 5 min. At last, the absorbance value at 545 nm was measured using a UV–Vis spectrophotometer (Perkin Elmer, USA). To determine the hemolysis rate of various samples, 0.2 mL of diluted blood was added to deionized water and 0.9% physiological saline, which were used as positive and negative controls, respectively. This study was performed in triplicate, and the hemolysis rate was calculated as

follows:

$$\text{Hemolysis rate (\%)} = \frac{(A_s - A_n)}{(A_p - A_n)} \times 100 \quad (3)$$

where A_s, A_n and A_p represent the absorbance values of the samples, negative control group and positive control group, respectively.

Clotting time tests: APTT and PT were used to evaluate the anticoagulation property of the QCS/MFC cryogel. Firstly, QCS/MFC cryogels of 15×15×15mm were immersed in PBS with 24-well plates overnight, and then incubated at 37 °C for 1 h. After removing PBS, 100 μL fresh platelet-poor plasma (PPP) was introduced and incubated at 37 °C for 20 min. PPP was obtained by centrifuging the whole anticoagulant blood at 4000 rpm for 15 min. For APTT measurement, 100 μL of APTT agent was added to the wells at 37 °C and incubated at 37 °C for another 5 min. Then, 100 μL of 0.025 M $CaCl_2$ was added. Then, the coagulation time was measured as APTT value. To test PT, 100 μL Thromborel S (incubated 10 min before use) was added to the wells at 37 °C and further incubated at 37 °C for 2 min, and then the PT values were measured. Pure PPP without samples was the control group. All measurements were carried out three times.

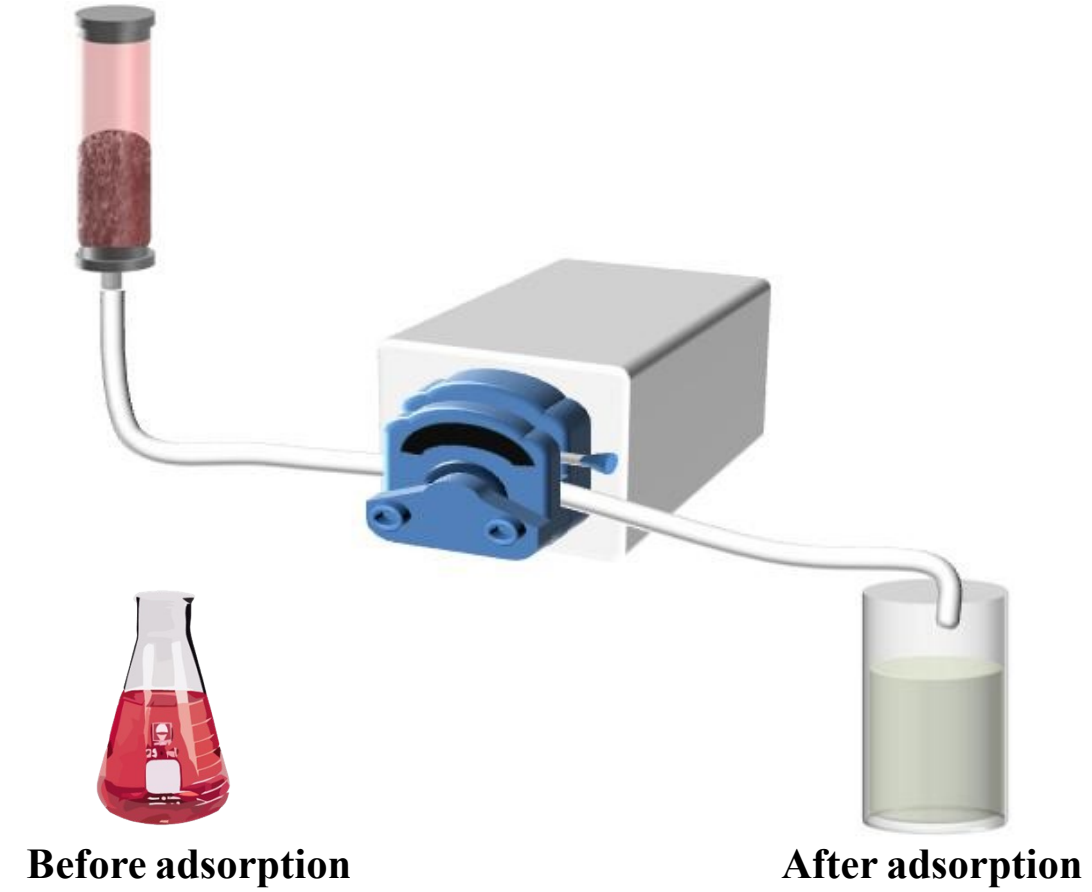

Figure S1. Schematic diagram of the self-made hemoperfusion device for simulating the dynamic adsorption process.

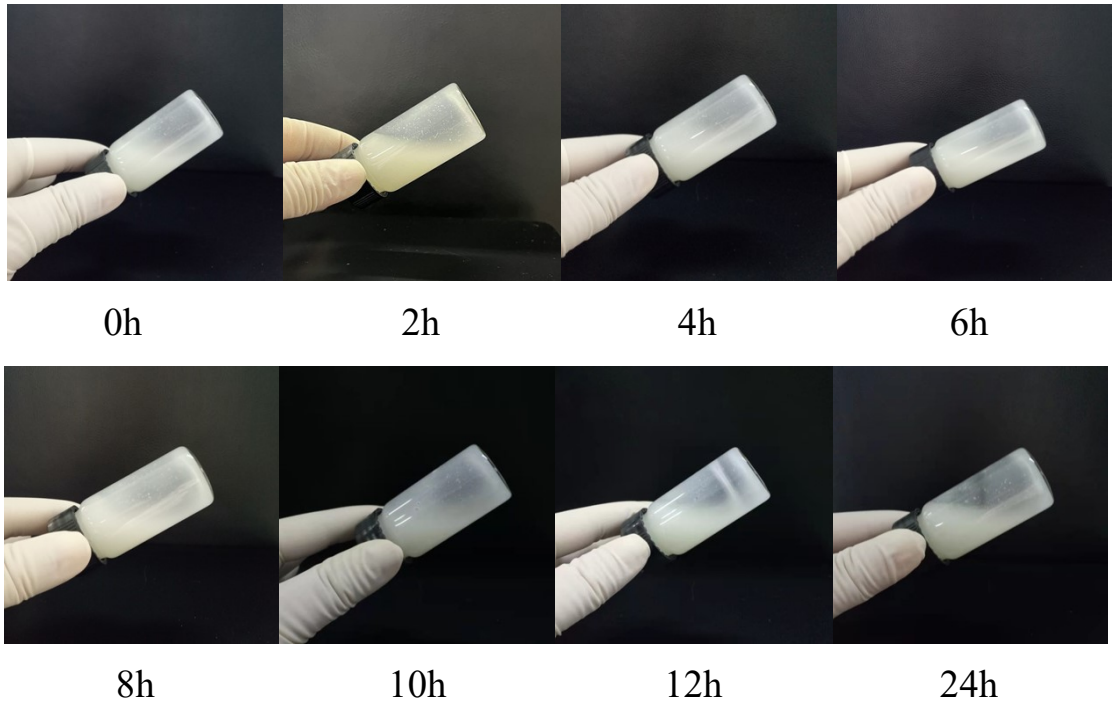

Figure S2. Photographs of QCS/MFC suspensions containing BVSM cross-linker for incubation at room temperature for 24 h, demonstrating no gelation at room temperature.

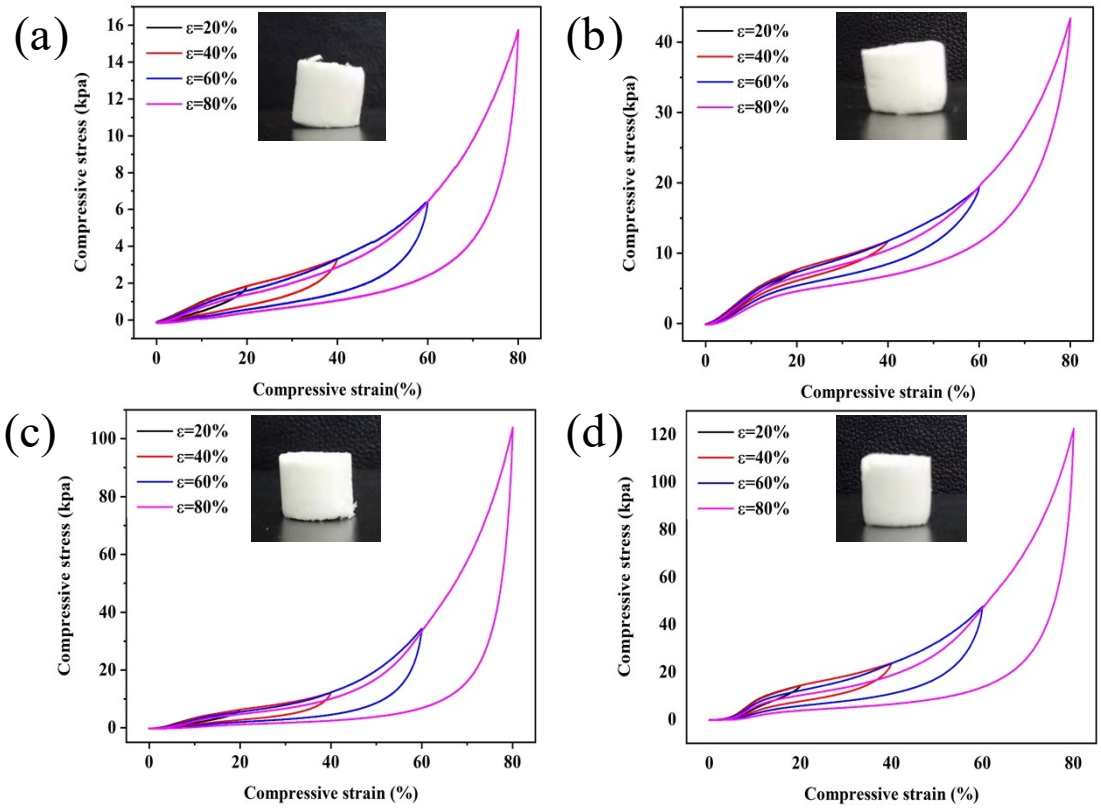

Figure S3. Underwater compression stress-strain curves at various strain settings for the QCS/MFC cryogels with various BVSM contents of (a) 1 wt%, (b) 1.5 wt%, (c) 2 wt% and (d) 3 wt%. Insets show the air-dried cryogels after alcohol solvent exchange.

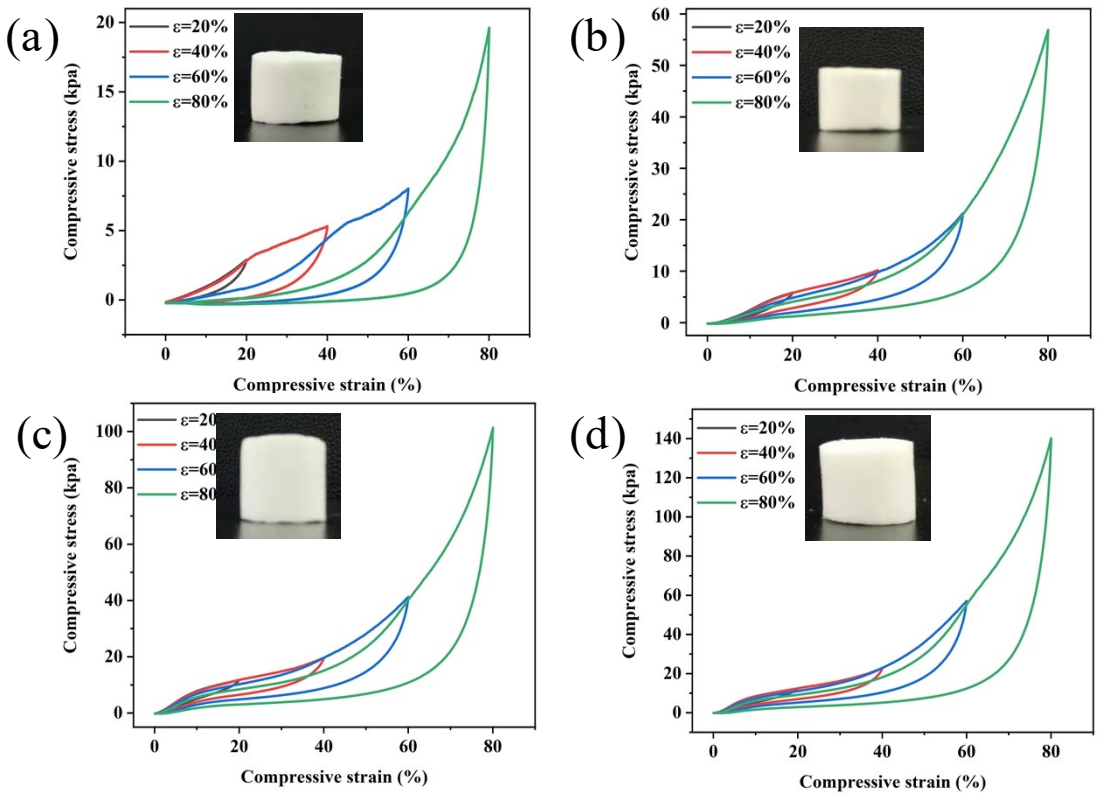

Figure S4. Underwater compression stress-strain curves at various strain settings for the QCS/MFC cryogels with various MFC contents of (a) 0.25 wt%, (b) 0.5 wt%, (c) 0.75 wt% and (d) 1.0 wt%. Insets show the air-dried cryogels after alcohol solvent exchange.

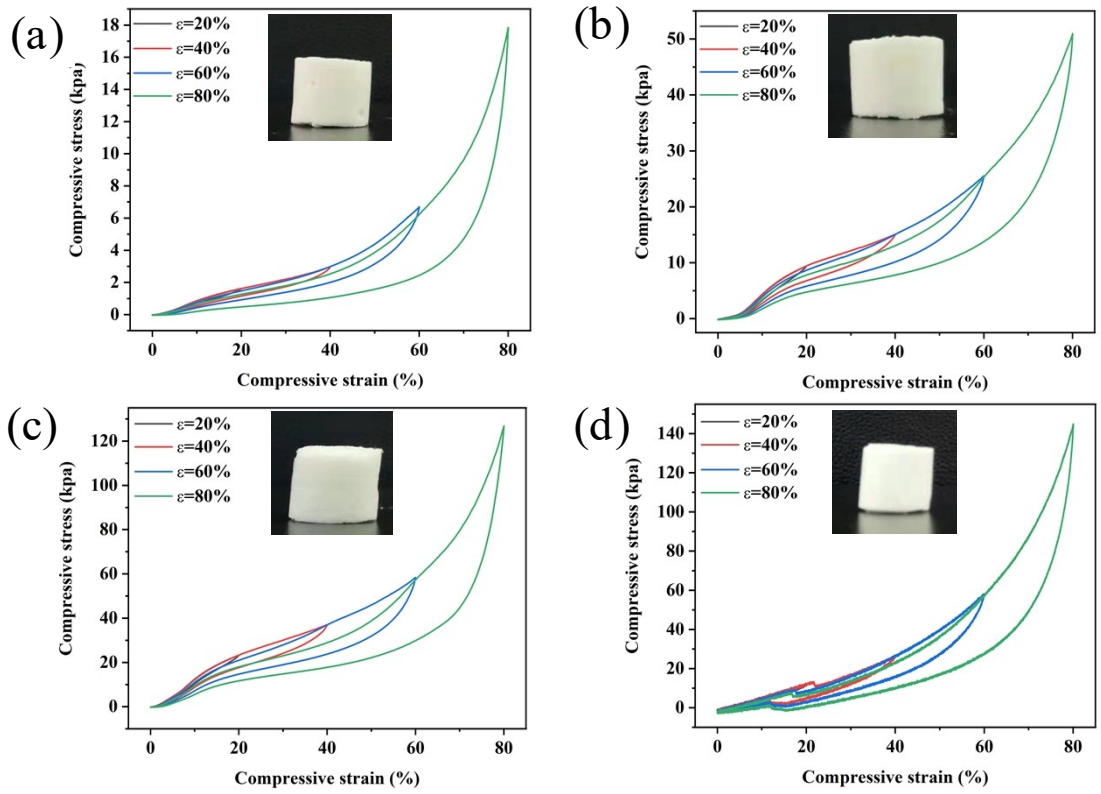

Figure S5. Underwater compression stress-strain curves at various strain settings for the QCS/MFC cryogels with various QCS contents of (a) 1.0 wt%, (b) 2.0 wt%, (c) 3.0 wt% and (d) 5.0 wt%. Insets show the air-dried cryogels after alcohol solvent exchange..

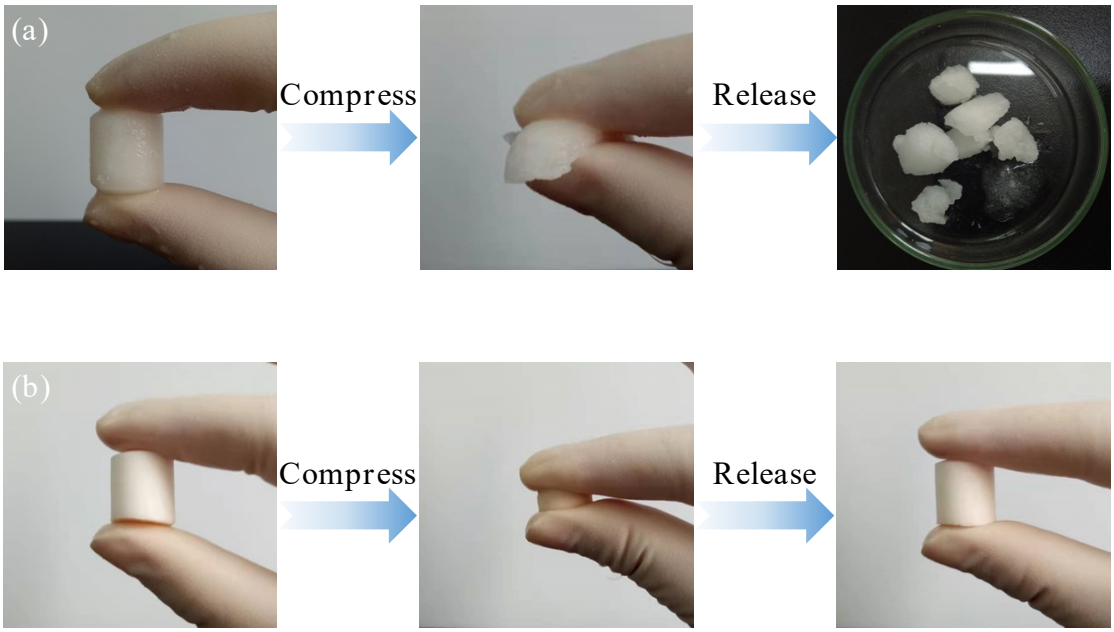

Figure S6. Photographs of pure QCS cryogel (a) and QCS/MFC cryogels (b) withstanding the compression in air, showing MFC influence on the shape recovery capacity of QCS/MFC composite cryogel.

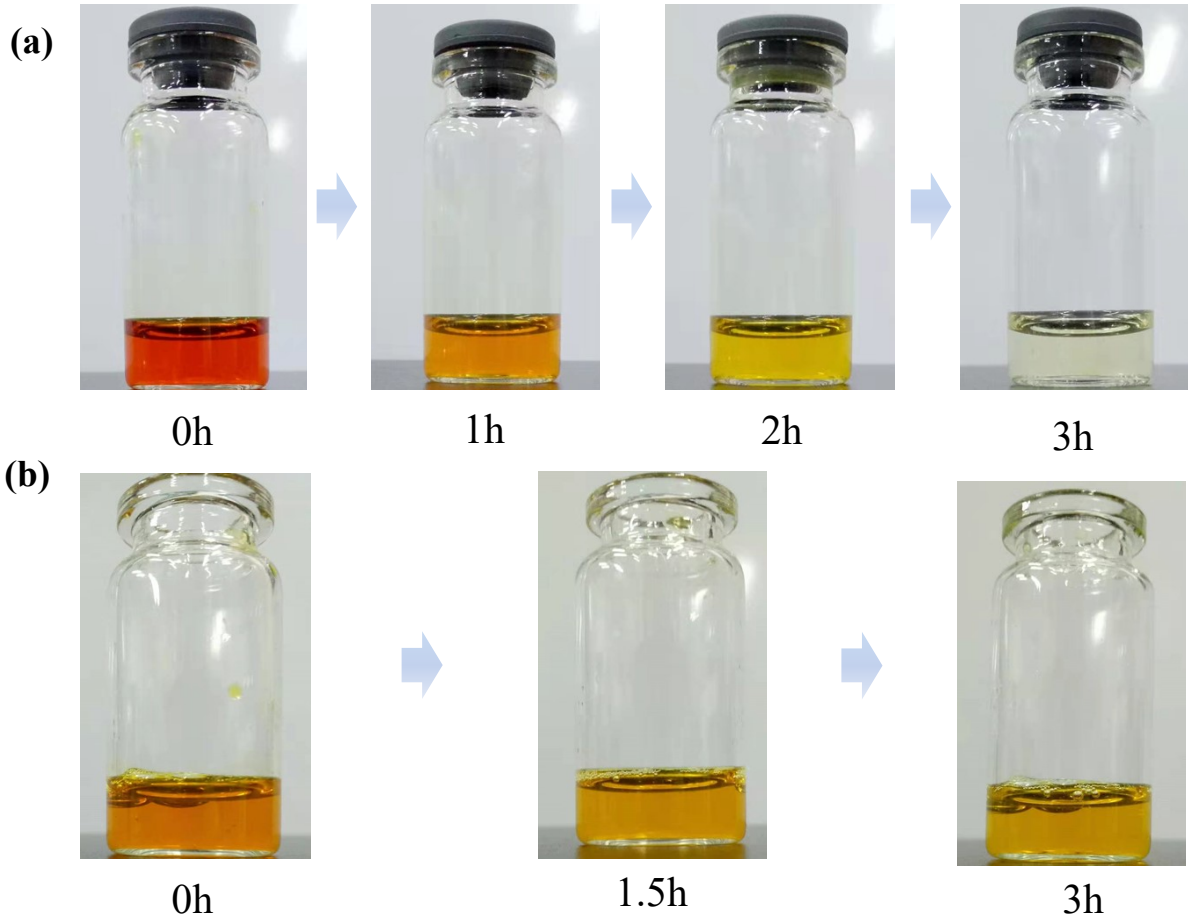

Figure S7. Photographs of bilirubin supernatants containing 0 (a) and 40 g/L BSA (b) after adsorption by the QCS/MFC cryogel for different time.

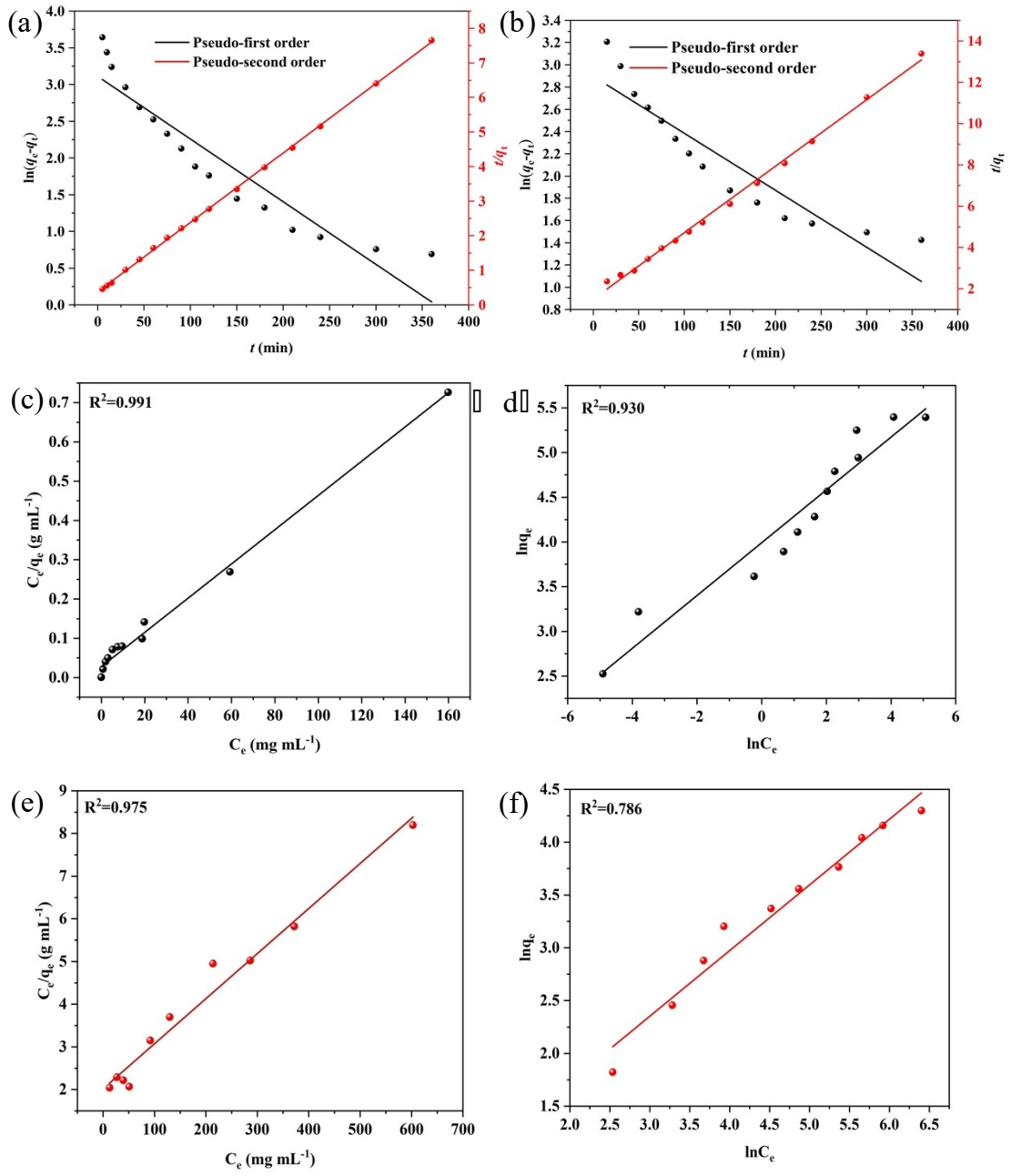

Figure S8. Linear fits using pseudo-first-order and pseudo-second-order models for bilirubin adsorption kinetics of the QCS/MFC cryogel in albumin-free (a) and albumin-rich (b) PBS solution. Bilirubin adsorption isotherms fitting curves of the QCS/MFC cryogel using linear Langmuir model for albumin-free (c) and albumin-rich (d) PBS solution, as well as linear Freundlich model for albumin-free (e) and albumin-rich (f) PBS solution.

Table S1. Bilirubin adsorption kinetic parameters of the QCS/MFC cryogel at the bilirubin concentration of 100 mg/L.

Albumin-free solution				
q_e	Pseudo 1st order		Pseudo 2st order	
(exp, mg/g)	q_e	R^2	q_e	R^2
47	46.7	0.889	47.5	0.999
Albumin-rich solution				
q_e	Pseudo 1st order		Pseudo 1st order	
(exp, mg/g)	q_e	R^2	q_e	R^2
29	27.45	0.979	28.92	0.994

Table S2. Intra-particle diffusion parameters for bilirubin adsorption on the QCS/MFC cryogel.

Albumin-free solution		Albumin-rich solution	
$K_{id,\,1}$ (mg g^{-1} $min^{0.5}$)	$K_{id,\,2}$ (mg g^{-1} $min^{0.5}$)	$K_{id,\,1}$ (mg g^{-1} $min^{0.5}$)	$K_{id,\,2}$ (mg g^{-1} $min^{0.5}$)
5.285	0.951	2.108	0.184

TableS3. The fitting constants and correlation coefficients obtained from Langmuir and Freundlich models for bilirubin on the QCS/MFC cryogel.

Albumin-free solution					
Langmuir model			Freundlich model		
Q_{max} (mg/g)	K_L (L/mg)	R^2	K_F ((g/mg)(L/mg)$^{1/n}$)	$1/n$	R^2
250	0.148	0.991	51.94	0.2276	0.930
Albumin-rich solution					
Langmuir model			Freundlich model		
Q_{max} (mg/g)	K_L (L/mg)	R^2	K_F ((g/mg)(L/mg))$^{1/n}$	$1/n$	R^2
100	0.00533	0.975	1.594	0.634	0.786